THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXII—No. 2

Issued October 1, 1922

BALTIMORE, U. S. A.

1922

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

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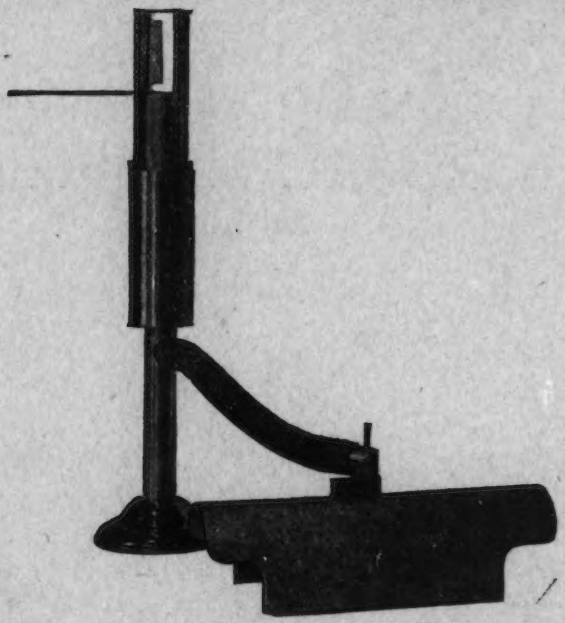

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VOL. 62 OCTOBER 1, 1922 No. 2

VASCULAR REACTION TO EPINEPHRIN IN PERFUSATES OF VARIOUS C_H

II. The Portal-Venous System of the Liver

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Received for publication July 11, 1922

In a paper by Snyder and E. Cowles Andrus (1), some evidence was given indicating that the hydrogen-ion concentration of a perfusing fluid may be the factor that determines whether a minimal dose of epinephrin will have an exciting or a depressing effect upon the smooth muscle elements in the heart, that is, whether the tonus increases or decreases. This observation led Snyder and Campbell to a series of experiments upon smooth-muscle structures to determine whether a similar reversal effect of epinephrin could be obtained by small changes in the C_H of the surrounding media. One set of experiments reported upon briefly by these authors (2) gives some results on perfusing the vascular system of frogs by Trendelenburg's method. Here it was found that if adrenalin-Takamine was used in minimal active doses then its effect could be made exciting or depressing at will by simply changing the C_H of the perfusing fluids. If the pH of the fluid was made 7.8 this of itself would have, as is well known, a constricting effect but if in addition adrenalin-Takamine were present in the ratio of 1 part to 10^9 parts of the fluid then constriction became still greater. On the other hand if the pH of the perfusing fluid was brought down to 7.0 or 7.2 then the dilating effect of this fluid could be increased by adding to it the drug in the ratio of $1:10^9$.

In these preliminary studies it was further found that the reversal could sometimes still be got if the pH indices of the two fluids were set as

close as 7.3 and 7.6. (For this extreme sensitivity of tissue to pH changes see also E. Cowles Andrus (3).) The objection may still be raised that these concentrations of H-ions do not obtain in the blood and therefore there can be little point in experimenting with them. The answer to this is that we only know the C_H of blood taken from the main vascular channels. We do not yet know the C_H of capillary blood and much less of the lymph of localized areas, or of cell plasmas, under various phases of tissue activity. The probability is that the C_H varies considerably at different points and at different times. If this is the case then it is clear that upon mixing in the main streams the OH- and H-ions of various concentrations quickly neutralize each other thus establishing the C_H that has been so often observed for blood and that has been even lifted up of late to the plane of a datum of universal significance. But it is in the sharply localized areas where the special activities of the body take place, and it is in these areas that we must look for answers to most of the fundamental problems of physiology. In the light of these considerations it can be judged no longer a futile task to inquire further into the behavior of organs when perfused with fluids whose C_H deviates from that of the main blood stream.[1]

One of the organs of the body of chief interest in connection with the rôle of the hormone of the medulla of the adrenals is the liver. The present paper accordingly deals with the results of perfusing the terrapin's liver with saline solution of various C_H, with and without minimal amounts of the Takamine preparation.

The immediate object of the present experiments was to determine the effect of these solutions upon the rate of outflow from the hepatic veins with the inflow cannula inserted into the portal vein, and with the hepatic arterial supply cut off.

In general practice the terrapins were pithed as to brain and upper part of spinal cord. The ascending vena cava and the coronary vessels of the stomach were ligated. The portal vein in this animal before entering the liver divides into two principal branches, one of which supplies each of the two (right and left) main lobes. There were evident smaller veins joining the left portal branch, or the omphalic vein, directly from the stomach. For this reason the gastric vessels were tied

[1] It would be of interest in this connection to review the various degrees of C_H of the media in which many microörganisms are able to live, and in which they live best; but less far removed are the bizarre deviations of C_H to which the normal fluids of the human body subject certain groups of cells, of the gastric mucosa, e.g., and that of pancreatic ducts.

to prevent an uncontrolled seepage into the liver. The vessels leading blood from liver to sinus in the terrapin consist usually and chiefly of a large vein from each of the two main lobes each joining the sinus venosus directly. Sometimes additional smaller hepatic veins are seen also joining the sinus directly. By inserting a wide outflow cannula into the sinus at the gateway to the atria, with the two descending venae cavae tied at their junction with the sinus, with the ascending vena cava tied as high up as it could be reached without injuring the liver, with the gastric vessels tied off and the inflow cannula in the portal vein,the liver of the terrapin is almost completely isolated from the rest of the body. There may be still some small collaterals from the short length of the post-cava that could not be reached but this must be insignificant for in the successful preparations the rate of out-flow kept pace with the inflow to a remarkable degree.

The solutions were made up of glass re-distilled water containing 0.6 per cent NaCl, 0.03 per cent KCl and 0.02 per cent $CaCl_2$ to which was added minimal constant amounts of mono- and di-sodium phosphates in the proportions necessary to maintain the various concentrations of H-ion desired. Tests at the end of the experiments assured us that the C_H remained constant in the various perfusing bottles. These tests were made by the colorimetric method, using phenolsulphonephthalein against standard tubes.

The head of pressure of the perfusing fluids was kept constant by the use of Mariotte's bottles. This was set to equal about 10 cm. of a water-column, but the exact pressure together with room temperature and other variable data are noted in the tables exhibiting the results of the experiments. The solutions were put in four reservoirs the contents of two of which were adjusted to an H-ion of lower and that of the other two to an H-ion of higher concentration. To one of each of these two pairs adrenalin-Takamine was added to a concentration of about $1:10^9$.

One of the two adrenalin-free solutions, usually the one of lower pH, was used first to wash out the blood from the liver and to get the organ adjusted to the fixed conditions of the experiments. This was allowed to perfuse for several minutes. The method of recording the hepatic outflow was the drop-method. From the drops recorded the outflow for the various solutions could be reduced to a minute-volume denomination for comparison. In order to get a fair record of the effect of a new solution it was necessary to allow the fluid to perfuse the liver from 4 to 7 minutes before recording. The duration of the record itself

was about 2 minutes. After the perfusions were in progress for 1½ to 2 hours the usual edema characteristic for a Ringer's solution made its appearance, when the experiment was discontinued.

In all, ten animals were experimented upon. Of these four showed some signs of disease or of a moribund condition, and the blood vessels

TABLE 1

DATE OF EXPERIMENT AND REMARKS	SEQUENCE OF PERFUSING FLUIDS	CHARACTERISTICS OF PERFUSING FLUIDS		VOLUME OUTFLOW NUMBER OF DROPS PER MINUTE
		pH index	Adrenalin	
December 23, 1921. *ca* 5 minute duration for each solution. Pres. 15 cm. of water. Temp., 21°C. Left lobe only perfused.	1	6.8	0	102
	2	6.8	+	150
	3	6.8	0	138
	4	6.8	0	120
	5	6.8	+	132
	6	6.8	+	126
	7	7.7	0	42
	8	7.7	+	30
January 5, 1922. 5 minutes duration for each solution; 11 cm. of water pres; temp. 21°C.	1	6.8	0	78
	2	6.8	+	102
	3	6.8	0	80
	4	6.8	+	102
	5	6.8	0	90
	6	6.8	0	90
	7	7.7	0	30
	8	7.7	+	12
January 7, 1922. *Ca* 6 minutes duration for each solution; pres. 12 cm. water; temp. 20°C.	1	6.8	0	66
	2	6.8	0	66
	3	6.8	+	78
	4	6.8	0	52
	*5	7.8	0	12
	6	7.8	+	6
	7	6.8	0	8
	8	6.8	0	78

* Between these two a longer interval of time (about 12 minutes instead of 6) intervenes. The experiment was disturbed by some reflex movements of the limbs.

reacted poorly in one way or another. These were thrown out of consideration. The other six however gave excellent responses and the protocols indicate that the experiments technically were faultless. The results of these all indicate that, as in the terrapin heart and in the

vascular system of the frog, here too the terrapin's portal-venous system shows beyond a doubt the reversal effect of epinephrin.

The chief results of four experiments are shown in the following tables, and will suffice to demonstrate the point. The legends explain what the figures stand for. It may be added that the zeros under the subheading "adrenalin" indicate that none of the drug was added to the fluid used in that perfusion, whereas the plus signs indicate the addition of the drug in the proportion $1:10^9$. It should be further added that the tables for January 5 and 21 do not contain all the perfusion results. In the case of the experiment for January 5 there were 21 changes of solutions all of which except three give results in the direction of the eight cases shown in the printed table. Two of these exceptions are explicable if one supposes that there is a certain lag, that more time to wash out the previous solution from the tissue ought to have been given. In the third case adrenalin added to the solution of the higher pH actually gave an increase in outflow! We have no explanation for this at all. In the case of the experiment for January 21 there were 13 changes of perfusing fluids and fifteen records during an hour and forty minutes, all of which gave results of the same character as those shown in the printed table. Still later changes in the perfusates gave less clear-cut results, evidence of edema began to appear and the minute-volume outflow was reduced to nearly half the original.

Experiment of January 21, '22. Right lobe of liver only perfused with simple Ringer's pressure, 10 cm. of water; temperature, 21°C; duration of each solution, 7 minutes. Adrenalin chlorid, when added, in concentration of $1:10^9$

SEQUENCE OF PERFUSION	CHARACTERISTICS OF PERFUSING FLUIDS		VOLUME OUTFLOW IN DROPS PER MINUTE
	pH index	Adrenalin	
1	7.8	0	28
2	7.8	+	2
3	6.8	0	31
4	6.8	+	50
5	6.8	0	31
6	6.8	+	35
7	7.8	0	16
8	7.8	+	4
9	7.8	0	19

SUMMARY AND DISCUSSION

These experiments together with those previously reported (1), (2), show conclusively that, with the H-ion concentration of a perfusing fluid set somewhat above that of the vascular blood of animals, epinephrin when given in minimal effective dosage is an inhibiting or a depressor agent. With the C_H set somewhat below that of the blood of the general circulation epinephrin given in the same minimal dosage still has the exciting or pressor action that is observed invariably for larger dosages. This conclusion is based upon experiments only in the isolated heart and the hepatic portal-venous system of terrapin, and the vascular system of frogs. It is of interest however to inquire what bearing these results have upon the previous observations of a depressor action of epinephrin on general blood pressure as reported and given considerable study by Cannon and his pupils (4). According to these authors depressor phenomenon in mammals has been observed from time to time since 1899 by various investigators. Among them all several explanations have been offered. While these explanations are interesting and plausible none of them has received sufficient proof to be entirely acceptable. There is one line of thought, however, that the present results suggest, that ought to be given careful consideration. Under the usual conditions of mammalian experimentation there is almost invariably some degree of asphyxiation. If the C_H of the blood in the large vessels does not show an increase, yet the known inadequate oxidation and removal of acid metabolites would make it seem probable that the reaction of the tissue at least may have suffered some such change. In this case, arguing from the findings in this laboratory as described above, it would seem that a more specific cause for the reversal effect of epinephrin can now be entertained. Collip (5) experimenting with dogs under ether and chloroform was able to get well-marked depressor responses to epinephrin if the animals were under light anesthesia. By increasing the depth of anesthesia the depressor was converted into a pressor effect. Further, the depressor responses during light anesthesia could be converted into a pressor response by administering enough $NaHCO_3$ to raise the pH of the blood. Again the pressor response during deep anesthesia could be distinctly lessened by injections of acid sodium phosphate. These experiments clearly confirm the idea that the reversal or opposed action of epinephrin in great dilution may be a function of the C_H of the tissues and probably of the media immediately bathing them. For in the case of the experiments just quoted one may suppose that under light anes-

thesia the animals suffered some asphyxia, their tissues then taking on a higher C_H but that under deep anesthesia the general metabolism was lessened to the point where acid formation ceases, and that that had accumulated became oxidized or removed, the C_H falling. The usual pressor effect of the drug then prevailed.

We may sum up by saying that experiments in this laboratory on lower vertebrates have shown that changes in the H-ion concentrations of a perfusing fluid will determine whether a minimal dose of epinephrin shall be pressor or depressor, excitatory or inhibiting, in its action. This fact gives a clew as to why epinephrin administered to mammals at times has been observed to cause a fall of general blood pressure or an increase of volume of a part. The further observations of Collip on mammals strengthen this surmise as to the rôle of the H-ion.

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(5) Collip: This Journal, 1921, lv, 450.

COMPARATIVE STUDIES ON RESPIRATION

XXIII. The Effect of Adrenalin on the Production of Carbon Dioxide by Animals and by Plants

DOROTHY M. HUTCHINSON

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Received for publication July 12, 1922

The adrenalin used in these investigations consisted of the dry product obtained from Parke, Davis & Co. The dry powder was dissolved in hot water (95°C.) and rapidly cooled to room temperature to check the oxidation which proceeds rapidly at high temperatures (producing a pink coloration followed by a browning of the solution).

The production of CO_2 was measured by means of the apparatus described by Osterhout (1). The rate of respiration was taken as the reciprocal of the time required to change the indicator from pH 7.78 to 7.60 and is expressed as per cent of the normal (which is in all cases taken as 100 per cent).

The plant material consisted of radish seedlings with caulicles from half to three-fourths of an inch in length. The seeds were sterilized and allowed to germinate under nearly aseptic conditions on moist filter paper covered by a bell jar. If they showed any signs of mold they were discarded. Just before use they were washed for 10 minutes in running water. They were then placed in the apparatus in distilled water. After the rate of respiration had become constant the distilled water was replaced by a solution of adrenalin. Care was taken to avoid errors due to the opening of the apparatus. In order to avoid effects due to the admission of laboratory air and to absorption of CO_2 by the solution of adrenalin it was decided to discard the readings made during the first 10 minutes after the solution of adrenalin was added. Under the conditions of the experiment this seemed a sufficient safeguard, as shown by control experiments in which distilled water, without seedlings, was introduced. The buffer effect of the concentration of adrenalin here employed was negligible.

The first experiments were made with very dilute solutions of adrenalin, but as these produced little or no effect higher concentrations were

employed. With 0.003 per cent (0.000164M) the rate of production of CO_2 fell rapidly (see fig. 1) and at the end of 14 minutes was only 20 per cent of the normal. After this it gradually rose and at the end of 50 minutes had reached 74 per cent. It continued thus for an hour, after which the experiment was discontinued.

With 0.002 per cent (0.000109M) a similar curve was obtained. The rate fell to 40 per cent, then rose to 82 per cent and so continued for an hour, after which the experiment was discontinued.

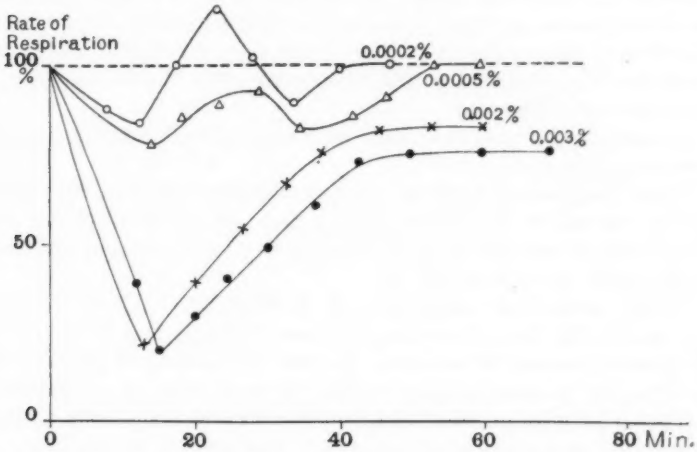

Fig. 1. Curves showing the rate of respiration (expressed as per cent of the normal) of radish seedlings in solutions of adrenalin. The normal rate (which is taken as 100 per cent) corresponds to a change from pH 7.78 to pH 7.60 in about 54 seconds.

Each curve represents the average of 10 experiments; probable error of the mean less than 2 per cent of the mean.

Since these effects were so pronounced weaker solutions were tried. The curves obtained were somewhat different. With 0.0005 per cent (0.0000273M) there was a drop to 78 per cent followed by a gradual rise which reached 92 per cent at the end of 29 minutes; it then fell to 81 per cent and rose gradually, reaching the normal at the end of 56 minutes.

A somewhat similar curve was obtained with 0.0002 per cent (0.0000109M), the rate falling to 82 per cent in 13 minutes and then rising to a maximum of 116 per cent, after which it fell to 86 per cent and then rose to the normal.

In order to compare these results with those obtained with animal tissue experiments were made with the muscles of winter frogs. The entire hind leg was used, after carefully removing the skin. The muscles were placed in the apparatus in Ringer's solution and measurements were made at frequent intervals. It was found, in agreement with the results of Fletcher (2), that the rate fell for about 2 hours but that after this the drop was so slow as to be negligible for the purposes of these experiments. The procedure, therefore, consisted in allowing the muscle to remain in the apparatus (in Ringer's solution) for about 2 hours and then replacing the Ringer's solution by adrenalin dissolved in Ringer's solution. As in the case of radish seedlings, the readings taken during the first 10 minutes after the introduction of adrenalin were discarded.

In order to compare the results with those already described similar concentrations were used.

With a solution of 0.002 per cent (0.000109M) the rate fell rapidly and at the end of 15 minutes had reached a minimum of 15 per cent after which it rose slowly for 45 minutes to 63 per cent and continued at this point for an hour (fig. 2).

With a solution of 0.0005 per cent (0.0000273M) the rate fell to 70 per cent in the first 12 minutes but rose gradually and at the end of 24 minutes reached 97 per cent. It then fell again to 87 per cent and at the end of 45 minutes it had reached the normal rate of respiration.

With a solution of 0.0002 per cent (0.0000109M) the rate dropped slowly and at the end of 22 minutes it had reached 68 per cent. It then rose to normal at the end of 32 minutes, after which it dropped to 82 per cent but came back to normal at the end of 60 minutes.

With a solution of 0.0001 per cent (0.0000055M) the rate fell to 68 per cent but rose to a maximum of 103 per cent at the end of 24 minutes, falling again to 79 per cent, and rising to normal at the end of 50 minutes.

A comparison of the curves obtained with animal and plant material shows a striking similarity. In both cases the stronger solutions depress the rate in marked fashion. The fact that this is followed by a return toward the normal is due in part to the oxidation of the adrenalin, for when the curve has passed the minimum and is returning toward normal we find that if the solution of adrenalin is replaced by a fresh solution the curve at once falls and after a time begins to rise again. This is regarded as an indication that the adrenalin was beginning to lose its efficiency as the result of oxidation (in many cases this is evidenced by a pinkish coloration).

The curves obtained with weaker solutions are peculiar in exhibiting two minima. It is possible that the rise after the first drop might be explained as due to the oxidation of adrenalin, as mentioned above, but this cannot apply to the second minimum unless we suppose that the second drop is due to new substances formed by the process of oxidation.

It was found in the case of radish seedlings in 0.0002 per cent adrenalin that if the adrenalin was replaced by a fresh solution just before the curve reached the second minimum it did not rise to the normal

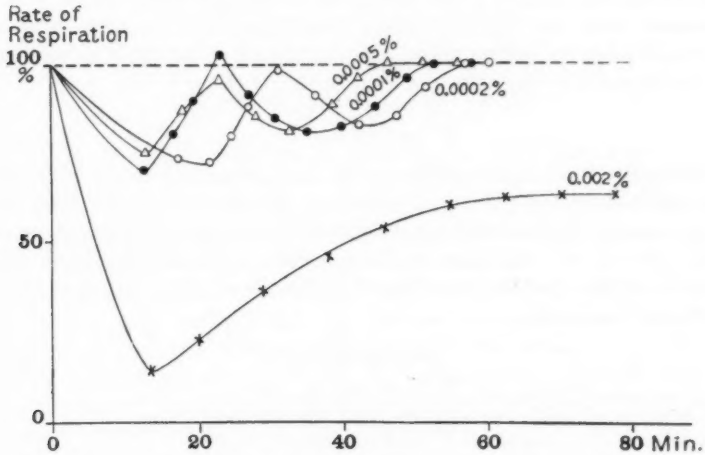

Fig. 2. Curves showing the rate of respiration (expressed as per cent of the normal) of frog muscle in solutions of adrenalin. The normal rate (which is taken as 100 per cent) corresponds to a change from pH 7.78 to pH 7.60 in from 1.5 to 3.25 minutes, depending on the material.

Each curve represents the average of 10 experiments; probable error of the mean less than 3 per cent of the mean (except at three points, where it is less than 5 per cent).

and remain there but, on the contrary, rose above the normal (to 120 per cent), then dropped to 90 per cent, and finally rose to the normal and remained there. This indicates that rhythmical chemical processes are going on, probably as the result of the oxidation on the adrenalin.

After this paper was written there appeared an article by Martin and Armitstead (3) in which it is stated that the respiration of frog's muscle may be increased as much as 25 per cent by very dilute solutions of

adrenalin (0.000235 per cent) and as much as 400 per cent by solutions of 0.005 per cent to 0.01 per cent. Martin and Armitstead do not mention a depression of the rate in any of the solutions employed by them. Inasmuch as they added the indicator directly to the solution containing the muscle it would be affected by acids other than carbonic, and if such are produced by the muscle its rate of respiration would appear greater than it actually is. They do not mention whether they took precautions to avoid an error of this sort. In the experiments of the writer non-volatile acids cannot affect the indicator (which is in a separate tube) and under ordinary conditions such a volatile acid as acetic will have no effect unless the concentration is very high and the exposure much longer than in the experiments of the writer.

SUMMARY

Adrenalin has similar effects on the respiration of frog's muscle and of radish seedlings. Stronger solutions (0.002 per cent to 0.003 per cent) cause a depression: this is followed by a return to normal, probably due to the oxidation of the adrenalin. Weaker solutions produce a rhythmic effect: the rate of production of CO_2 falls, rises, then falls and rises again.

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STUDIES OF THE THYROID APPARATUS

VII. A Differential Effect of Thyro-Parathyroidectomy and Parathyroidectomy on the Incisor Teeth of the Albino Rat

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Received for publication July 13, 1922

In the preceding article of this series (1) there was reported the fact that the complete removal of the thyroid gland with the parathyroids produced but little if any effect upon the weight of the submaxillary glands of the albino rat, while parathyroidectomy alone caused a considerable increase in the size of this organ.

This paper is concerned with a similar differential effect in which the incisor teeth are involved. It has been known for some time that the removal of the parathyroid glands from the albino rat results in serious disturbances in growth of the incisor teeth. Detailed studies of the dental defects produced under such circumstances have been reported by Erdheim (2), (3) and Toyofuku (4).

During the course of our investigations, the general plan of which has been outlined in the preceding paper (1), it has been observed that dental defects follow parathyroidectomy with great frequency. On the other hand when the thyroid is removed together with the parathyroids these disturbances do not appear. It is our purpose to emphasize this difference and to present a tentative interpretation of the phenomenon.

There are various types and degrees of dental defects found after parathyroidectomy. The least harmful disturbance consists of the change from the normal pearly semi-translucency to a pathological snowy-white opacity, already noted by Erdheim. With this change there may or may not be partial or complete loss of the incisor pigment. More serious disturbances may show themselves as a kind of overgrowth, particularly of the upper incisors, in which they grow to exceeding great length and curling around frequently reach and may even penetrate

the roof of the mouth. In other cases one or more incisor teeth may have become so fragile as to break off, either below or above the gum. The residual fragment is usually found to consist of a mere shell of calcareous matter, hollow and quite brittle. In the accompanying drawings there are shown two specimens of the breakage and overgrowth defects described above and for comparison the normal condition. Drawing *3* was made from a thyro-parathyroidectomized rat 150 days of age. Drawings *1* and *2* were made from rats which had been

Fig. 1. Showing the type of dental defects in the incisor teeth of albino rats after parathyroidectomy compared with teeth from a thyro-parathyroidectomized rat. *1* and *2*, Teeth defects after parathyroidectomy. *3*, Teeth from thyro-parathyroidectomized rat. (No different from normal.)

parathyroidectomized at 75 days of age and which were dissected at 150 days of age, the time when the pictures were made. It should be noted that the teeth of the thyro-parathyroidectomized rat do not differ from those of the normal control rat in all outward appearances.

In table 1 there is given the relative incidence of the severe dental disturbances in a series of rats which were thyro-parathyroidectomized or parathyroidectomized at 75 days of age and carried through to dissection at 150 days of age. The controls were litter controls in all cases.

It is seen from this table that while but 1 out of the 20 thyro-parathyroidectomized animals showed disturbances of teeth growth some 20 out of the 26 parathyroidectomized rats gave evidence of severe dental defects. None of the controls had teeth disturbances.

Now the fragility and the hollowing out of the teeth indicate a serious disturbance in the calcium metabolism of these structures. That this represents a more or less generalized disturbance of calcium metabolism is evident from the findings of MacCallum and Voegtlin (5) of a decrease in the calcium content of the blood and a possibly greater excretion of calcium in parathyroidectomized animals. Since the work of these investigators, others have extended and confirmed the hypothesis of a severe disturbance of calcium metabolism as a result of parathyroidectomy. As stated in another place (6), I am not at present inclined to the opinion that a function of the parathyroids is the regulation of calcium metabolism, but rather consider that

TABLE 1

The relative incidence of dental defects in thyro-parathyroidectomized and parathyroidectomized albino rats and their litter controls. (Rats operated at 75 days and dissected at 150 days)

	THYRO-PARATHYROIDECTOMIZED	PARATHYROIDECTOMIZED	CONTROLS
Number of rats used	20	26	34
Number of rats with dental defects	1	20	0
Per cent affected	5.0	76.9	0.0

the disturbances in mineral salt balance observed after the loss of the parathyroid secretion are secondary changes incident to the tetany. However, the question I wish to consider here is why the loss of the parathyroid secretion causes these marked dental defects and the overgrowth of the submaxillary glands when the thyroid remains *in situ*, while when the thyroid is also removed no such results occur.

It has been quite nicely demonstrated by the combined studies of Plummer and Boothby (7) and Kendall (8) that a function of the thyroid gland is the production of a secretion containing as its active principle thyroxin (9) which acts in a way to maintain the tissues of the body as a whole at an adequate level of general metabolism. When the activity of the thyroid is increased the metabolism is increased, when it is lessened or destroyed the level of metabolism is lowered. Thus general metabolic stimulation is an important function of the thyroid.

From the present evidence the important function of the parathyroids is not stimulation, but rather inhibition, for when these glands are removed there is obtained every indication of a profound increase in neural irritability. There is as yet no evidence that this inhibition is of a directly general nature in the sense that the stimulation of metabolism by the thyroid secretion is. Nevertheless the indirect effects are general. A probable function of the parathyroids is the prevention, by some means or other, of the accumulation in the organism of certain toxic guanidine compounds that are known to be strong neural excitants. Whether or not these compounds directly stimulate general cellular metabolism is still a problem for investigation. But that they are at least indirect stimulators by virtue of their toxic action on the nerve endings is a certainty. As far as we now know, then, the function of the thyroid is in general directly stimulative, while the function of the parathyroids is more specificially inhibitive of a certain phase of nitrogenous metabolism, the by-products of which may give rise to general stimulation when not taken care of by the parathyroid secretion.

Now when the parathyroids are removed this stimulation effect, which may be acute and produce death with tetany, or chronic and allow of survival, is released. When the thyroid is left *in situ* and the parathyroids are removed there is thus added to the stimulative action of the thyroid the stimultive action of the products resulting from the loss of the parathyroid secretion. There is thus a summation of stimulation of metabolism. One of the results of this summation is a marked disturbance in calcium metabolism which gives rise to dental defects. Another is the enlargement of the submaxillary glands (1). On the other hand, when the thyroid and the parathyroids are both removed these peculiar expressions of metabolic disturbances are not seen. The loss of the thyroid secretion has resulted in a lowering of the plane of metabolism in general. The loss of the parathyroid secretion has resulted in the production or accumulation by the organism of products which indirectly stimulate metabolism. The algebraic sum of these phenomena yields to all outward appearances a metabolic unity, in which the disturbances of metabolism are not sufficiently marked to result in decalification of the incisor teeth or enlargement of the submaxillary glands. Such is our present interpretation of the differences in effects produced by parathyroidectomy and thyroparathyroidectomy.

These results may appear to be contradictory to the report in the first paper of this series (11) that the mortality rates are identical whether parathyroidectomy or thyro-parathyroidectomy is done. They are actually not so, since in those experiments we were dealing with acute effects of the loss of the parathyroid secretion, while here we are dealing with mild and chronic effects in animals so constituted as to withstand the loss.

In general it is our present opinion that the apparently specific effects (dental defects; submaxillary enlargement) following parathyroidectomy and which do not follow thyro-parathyroidectomy are peculiar expressions of a summation of stimulation of metabolism. They seem to indicate that the parathyroid function is in some way connected with calcium metabolism, but they do not indicate that this connection is direct.

These results do not necessarily imply that the thyroid and parathyroid functions are specifically complementary, supplementary or compensatory. There is no evidence to support a belief that the parathyroid function is specifically inhibitive directly of general metabolism. There is evidence supporting the belief that a thyroid function is specifically to stimulate directly general metabolism. That they are related as are all the functions of all the organs of the body is of course undebatable, but that the function of the one is specifically antagonistic to the function of the other is a view still without evidence to support it.

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ON THE PERMEABILITY TO DYESTUFFS OF THE PLACENTA OF THE ALBINO RAT AND THE WHITE MOUSE[1]

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Received for publication July 14, 1922

The problem of fetal nutrition is as yet inadequately interpreted. There is no doubt that the maternal organism digests and prepares the food, excretes the waste and regulates the physical conditions necessary for the development of the embryo. Set in the path traversed by nutritive materials on their way to the new organism is the placanta, a complex organ mainly composed of vascular fetal chorionic papillae which are bathed in the large blood spaces of the decidual membrane of the mother. Although the fetal and maternal bloods are separated from each other by the epithelial layers of the chorionic villi, nevertheless an active exchange of substances occurs, the mechanism of which is still incompletely understood.

The exchange of gases, water, sugar, salts, etc., is believed to be largely controlled by the physical processes of osmosis and diffusion. However, we are far from having data that would justify us in supposing that the exchange of all materials necessary for the growth of the fetus is the effect of these physical processes alone.

The proteins, which are colloids and undialyzable, present the first difficulty to such an explanation. In the case of these substances, so far as our present knowledge goes, we are obliged to assume that the epithelium of the chorionic villi is active by virtue of some specific function which cannot as yet be wholly explained, and further investigations are therefore required.

In a consideration of the permeability of the placenta we naturally turn to observations on the permeability of the cell membrane which has been extensively studied.

[1] This study was begun in the Zoölogical Laboratory of the University of Chicago and continued at The Wistar Institute of Anatomy and Biology in Philadelphia. Here I desire to express my thanks to Prof. F. R. Lillie, who suggested this problem and gave me the best of laboratory facilities. At the same time I wish to acknowledge my obligation to Prof. H. H. Donaldson for his helpful suggestions and criticism.

Among the numerous investigations which bear most directly on the problem of the permeability of the placenta by proteins are those which have been along the line of vital staining, because the majority of the dyestuffs used form colloidal solutions. From this point of view the vital dyes are suitable material for an investigation aiming at a study of the semi-permeable nature of the placenta. In 1905 Hofbauer published a monograph on the placenta in which all the previous studies with respect to its permeability to dyes are described and discussed. Since that time although staining has been so actively studied in connection with both cytology and chemotherapy, there has been, so far as I know, but little if any systematic study of the permeability of the placenta for dyestuffs.

Attention has been given largely to the staining of the granules in the cells, yet the dyes passing to the fetus through the placenta may be present in the fetal fluids without staining the cell granules. Thus we are justified in saying that our present knowledge concerning the permeability of the placenta for dyestuffs is incomplete.

For these reasons I have investigated the permeability of the placenta for a number of dyestuffs, aiming to get some information about the passage of colloidal material through the placenta, and have obtained certain results which seem worth reporting.

General methods. As experimental animals I used the pregnant albino rat and white mouse at or near full term, and made parallel tests on these two species. The solution of each dye was usually injected hypodermically between the scapulae. At varying intervals from 5 to 48 hours after the injection, the fetuses were removed by Cesarean section under light ether narcosis and the coloration of the mother and fetuses was determined. All placentae were thoroughly examined for pathological changes. If any abnormality was found the animal was eliminated from the series.

The dyes used in my experiments were chosen from those available at The Wistar Institute in Philadelphia and at the Zoölogical Department of the University of Chicago.

As is well known, some dyes undergo little or no chemical transformation when injected into the animal, while the others suffer definite chemical changes in the living body and may become colorless. The dyes belonging to the latter class are of no value for these investigations if they cannot be restored to their former colored state by later treatment. Furthermore, very few dyes are entirely devoid of poisonous properties, and some are very toxic, so that certain pathological changes will be

caused in organs and tissues (especially in the placenta) even if the animal survives, and thus interfere with the experiment. Accordingly much caution must be exercised in selecting the dye. In my experiments I used only those dyes which are easily soluble in water or in physiological salt solution and which stain the living animal without any serious ill effects.

Again, some of these dyes are eliminated very quickly, despite their ability to stain the animal, so that the animal once distinctly stained may become entirely free from the dye within a short time. When such were used I repeated the injection of the dye two or more times, to maintain the stained condition till the end of the test, i.e., the removal of the fetuses.

For the detection of the dye in the "leuco" state in the tissue, I employed various detection methods which will be described later. However we are justified in saying that the fetus is free from the dye when no color is detected in the fetus by the same method of detection that reveals the stain in the mother. For this reason I excluded the dyes which do not deeply stain the maternal body. The microscopic examination of the cell granules was not made because the granular stain occurs only when either the dye has a chemical affinity for certain elements of the cell or the dye solution is precipitated by certain physical characters of the cell, and if the dye stains the cell diffusely the minute size of the cell makes it hard to distinguish whether or not the dye is present in the cell. The twenty-three dyes used in these experiments are alphabetically enumerated in table 1.

Discussion. Of the twenty-three dyes here used, sixteen penetrated the placenta after their injection into pregnant animals, while the other seven were not found in the fetal body. The placenta was found to be permeable to alizarin-SO_3Na, Biebrich's scarlet, Bismarck brown, Bordeaux red, dahlia, eosin w. s., fuchsin (acid), indigo carmine, light green F S, methylviolet 6B, methylene blue, neutral red, Nile blue, orange G, safranin and toluidin blue, and non-permeable to alkali blue, anilin blue w. s., Congo red, isamin blue, lithium carmine, Niagara blue 2B and trypan blue.

All the former dyes were present in the fetal body within 12 hours after their injection into the mother. Bismarck brown had passed the placenta within 5 hours after injection. Although the minimal interval needed for each dye to color the fetus was not determined, it is not probable that all the effective dyes can penetrate the placenta as quickly as Bismarck brown. As mentioned in the protocols, methylene blue was

not found in the fetus 5 hours after the injection, nor was Bordeaux red detected in the fetus 6 hours after the injection.

In all cases when the placenta was not permeable the fetus was left in the maternal body at least 12 hours, sometimes 15 or 24 or even 48 hours, after the injection. It is unknown whether or not some of these dyes can pass through the placenta after longer intervals. It is well known that some dyes are excreted very quickly in the urine, or decomposed in the living body within a few hours, so that we cannot detect

TABLE 1

(G = Grübler)

1. Alizarin sulfonate of soda (G.)
2. Alkali blue (G.)
3. Anilin blue (water soluble) (G.)
4. Biebrich's scarlet (G.)
5. Bismarck brown (G.)
6. Bordeaux red (G.)
7. Congo red (G.)
8. Dahlia (G.)
9. Eosin (G.)
10. Fuchsin (acid) (The Harmer Laboratories, Phila., Pa.)
11. Indigo carmine (G.)
12. Isamin blue (G.)
13. Light green F. S. (G.)
14. Lithium carmine (Carmine—rub. opt. G.)
15. Methylene blue rectif. n. Ehrlich (G.)
16. Methyl violet 6B (G.)
17. Neutral red (G.)
18. Niagara blue 2B (National Anilin & Chemical Co.)
19. Nile blue hydrochlorate (G.)
20. Orange G (G.)
21. Safranin (G.)
22. Toluidin blue (G.)
23. Trypan blue (Presented by H. M. Evans to Dr. F. R. Lillie)

the color. In these cases repeated injections are necessary to continue the exposure of the placenta to the dyes. Such a repetition of the injection however is undesirable unless the dye is quite harmless. For this reason I took 12 hours as the standard interval for determining whether or not a dye passes through the placenta, and assumed that the dye which does not pass the placenta within 12 hours will not be able to do this in a longer interval.

No special tests were made of the relative intensity of the color given by the dye passing the placenta. However, if we compare by inspection

the intensity of the coloration of the fetus by each dye a general impression may be obtained of the relative amount which has passed.

Among the sixteen dyes to which the placenta was permeable, neutral red, orange G, methylene blue, safranin and toluidin blue stained the fetus to the greatest extent. The remainder of those passing through the placenta are arranged in groups II, III and IV in the order of their decreasing diffusibility—while those in group V do not pass at all.

TABLE 2

I. Group; dyes to which the placenta is most permeable
   - Neutral red
   - Orange G
   - Methylene blue
   - Safranin
   - Toluidin blue

II. Group; dyes to which the placenta is less permeable; less than group I
   - Bismarck brown
   - Eosin (water soluble)
   - Fuchsin acid
   - Dahlia

III. Group; dyes to which the placenta is less permeable than for those in group II
   - Light green F S
   - Biebrich's scarlet
   - Indigo carmine
   - Methylviolet 6B

IV. Group; dyes to which the placenta is least permeable
   - Alizarin-SO_3Na
   - Bordeaux red
   - Nile blue

V. Group; dyes which do not pass the placenta
   - Niagara blue 2B
   - Anilin blue
   - Trypan blue
   - Lithium carmine
   - Congo red
   - Isamin blue
   - Alkali blue

It is noteworthy that the permeability of the placenta for each dye was the same for the albino rat as for the white mouse. If the placenta of the rat allows the passage of a dye, then the placenta of the mouse allows it, and vice versa. In one sense this was to be expected but the striking similarity in the reactions strongly supports the accuracy of the results. Thus we feel certain that these results on the permeability of the placenta for the dyes tested is true for these two species, at least.

We naturally ask whether this selective property of the placenta for different dyes is purely a chemical or physical or physico-chemical phenomenon, or whether we have to search for some unrecognized physiological function of the placenta for such a selection. To the discussion of this very interesting question we now turn.

Among the twenty-three dyes here used eight are basic dyes and fifteen are acid. In the following table the dyes are arranged according to their acidic or basic character, and the permeability of the placenta for them is noted. The mark + means permeability of the placenta and the increasing number of these marks indicates an increase in permeability.

TABLE 3

Relative permeability of the placenta to the various dyes used, arranged according to their acidic or basic properties

ACIDIC DYES	PERMEABILITY	BASIC DYES	PERMEABILITY
Alizarin-SO_3Na	+	Bismarck brown	+++
Biebrich's scarlet	++	Dahlia	+++
Bordeaux red	+	Methylviolet 6B	++
Eosin w. s.	+++	Methylene blue	++++
Fuchsin acid	+++	Neutral red	++++
Indigo carmine	++	Nile blue	+
Light green F S.	++	Safranin	++++
Orange G	++++	Toluidin blue	++++
Alkali blue	−		
Anilin blue w. s.	−		
Congo red	−		
Isamin blue	−		
Lithium carmine	−		
Niagara blue 2B	−		
Trypan blue	−		

The mark − means that the placenta is not permeable to the dye in question.

If we examine table 3 we find the remarkable fact that all the basic dyes tested pass through the placenta. This result directs attention to Overton's lipoid theory ('99), i.e., that the permeability of the cell membrane for substances depends upon their solubility in fat or fatlike bodies. We know also that almost all basic dyes are lipoid-soluble. Therefore, so far as the basic dyes are concerned, though the number tested is not large, this lipoid theory for the permeability of the cell membrane would seem to be applicable to the permeability of the placenta. In explaining the permeability of the placenta for the acid

dyes however, the lipoid theory meets difficulties. Although a few of the acid dyes I used, as orange G and Bordeaux red, are known to be lipoid soluble (Ruhland, '08) and also pass through the placenta, the majority of acid dyes are lipoid-insoluble, yet many of them also pass the placenta. For instance, fuchsin (acid), indigo carmine and Biebrich's scarlet are lipoid-insoluble, yet they pass through the placenta.

So far as my results go it seems probable that the lipoid-soluble dyes, regardless of their acid or basic character, generally pass the placenta. At the same time it is also beyond doubt that many lipoid-insoluble acid dyes also can go through. Thus we see that the lipoid theory of Overton cannot completely explain the permeability of the placenta for dyes.

We turn next to the relation between the chemical constitution of dyes and their ability to pass the placenta. The studies on the permeability of the cell membrane by dyes have shown that the sulfonic acid group $R–SO_3H$ has a remarkable influence on the solubility of dyes and, consequently, on their penetrating power. It was generally believed sulfonic dyes did not permeate the cell membrane till Ruhland ('08) and later Küster ('11) reported a considerable number, as acid-fuchsin, Bordeaux red, orange G, indigo carmine, Biebrich's scarlet, etc., to which the cell membrane is permeable.

The majortiy of the acid dyes used in my experiments belong in this group. Trypan blue, Niagara blue, alkali blue, isamin blue, alizarin sulfonic acid soda, light green F S, fuchsin S, anilin blue w. s., orange G, Bordeaux red, Biebrich's scarlet, Congo red and indigo carmine are all sulfonic acid dyes. Among these thirteen sulfonic acid dyes, six have been shown to pass through the placenta, the remaining seven do not do so. From this fact we can see that the SO_3Na group in the molecule is not so important a factor for the passage of dyes through the placenta as it is believed to be for the penetration of the cell membrane. Moreover the number of the sulfonic acid groups in the molecule has no great value for the penetrating property of dyes, because mono-and disulfates are found not only among the dyes which pass, but also among those which do not. As to the azo-group $-N:N$, there is no indication that it is important in so far as affecting the penetrating ability of the dye is concerned.

Orange G, Bordeaux red and Biebrich's scarlet, all of which pass the placenta, and Congo red, trypan blue and Niagara blue, which do not pass through all belong to the azo-dye group. The basic dye, Bismarck brown, to which the placenta is permeable, is also an azo-dye.

Three dyes—alkali blue, anilin blue and isamin blue—which do not pass the placenta, belong to the triphenyl-methane group, while light green F S, fuchsin (acid) and eosin, to all of which the placenta is permeable, also belong to the triphenyl-methane group. Therefore it is probable that the radical constitution of the tryphenyl-methane group does not determine the passage of a dye through the placenta. Finally it is remarkable that all benzidin dyes tested in my experiments—Congo red, trypan blue and Niagara blue fail to pass the placenta. These three dyes are all known as good stains for the living cell. Schulemann ('12) reported an exhaustive investigation on the relation of the chemical constitution of the benzidin dyes to their vital staining property, in which he used more than two hundred dyes. His results show that the relation of the auxochrom to the chromophor determines not only the fundamental physico-chemical character, but also the formation of the compound between the dye and the reacting body-produces colored precipitates in the cell. To my regret, I could get only the three benzidin dyes above mentioned. Therefore I am unable to discuss the relation between the chemical constitution of the benzidin dye group and their power to pass the placenta, but so far as my experiments go, the benzidin azo-dyes seem unable to pass through the placenta.

One might think that the placenta has a mysterious power of selecting dyes which do not harm the fetus, and of preventing the passage of the toxic dyes, but this assumption is hardly acceptable. For instance, isamin blue and trypan blue are known as very slightly toxic dyes, if toxic at all, so that the animal can bear a series of the injections of any of these dyes without any notable ill effects and continue to grow and even to breed; yet these non-toxic dyes do not pass the placenta, while other more toxic dyes easily penetrate it.

As above mentioned, Schulemann, as I understand him, concedes the Ehrlich side-chain theory for vital staining. On the other hand he lays stress upon the physico-chemical character determined by the relation between the auxochrom and chromophor.

Höber ('08–'09) made investigations on the relation between the colloidal condition of the dye and its vital staining power. He examined the physico-chemical character of many dyestuffs and tried to explain the permeability of the cell membrane for the dyes from the side of their colloidal character, using the epithelium of the kidneys of the frog for the test. From this experiment he came to the conclusion that *a*, if a dye cannot be taken up by the epithelium of the kidney, then it is practically in a completely colloidal state; *b*, if a dye has a lesser tendency

to take on the colloidal state, then it is easily taken up by the epithelium. Such a conservative conclusion was a natural outcome of the fact that even highly colloidal dyes, as Congo red, Biebrich's scarlet, etc., were also taken by the epithelium of the kidney.

In 1911 Küster repeated Höber's experiment on plant cells in place of the kidney cells and came to the same conclusion as Höber. He also found a striking parallelism between the colloidal character of the dye and its power to penetrate the membrane of the plant cell. According to his results only the slightly colloidal or non-colloidal dyes are permeating, with a few exceptions; while Höber's result shows that the most of the colloidal dyes and some of the highly colloidal dyes penetrate the cell membrane of the kidney of the frog. If we pick out the dyes which were used in my experiment from the many dyes tested by Höber and Küster and arrange their results and mine side by side in a

TABLE 4

DYES	COLLOID CHARACTER OF DYE SOLUTIONS	HÖBER (ON KIDNEY CELL)	KÜSTER (ON PLANT CELL)	PERMEABILITY OF PLACENTA
Indigo carmine.....	Slightly or absent	+	+	+
Orange G*.....	Slightly or absent	+	+	+
Fuchsin (acid).....	Slightly or absent	+	+	+
Light green F S.....	Slightly or absent	+	+	+
Anilin blue w. s.....	Half colloidal	+	(Not tested)	−
Congo red.....	Highly colloidal	+	−	−
Biebrich's scarlet.....	Highly colloidal	+	±	+

* Höber used orange GG—Merck; Küster and I used orange G—Grübler.

table, we get the following. In the table + means a strong penetrability of the dye; ± means a weak penetrability; and − the cell membrane was non-permeable to the dye.

According to this table we see that the non-colloidal or slightly colloidal dyes generally pass through the placenta, as was to be expected. At the same time, however, it is remarkable that anilin blue w. s. which is defined by Höber as a "mittelmässig" colloid, is non-permeating, while Biebrich's scarlet, which is said by the same author to be a "stark" colloid, does permeate. Here we must remember that the colloidal characters of the solution of organic dyes are sometimes quite differently reported in the literature. While some authors count a solution as a real solution, others count it as a colloid. Such disagreements seem to be due to the difference of the method employed or to differences in the conditions at the time of determination. Höber himself already states

that the solubility of anilin blue w. s. is such as to yield a slightly colloidal solution, when it is judged by its precipitability by $CaCl_2$ or $NiCl_2$, while the ultra-microscopic appearance and diffusibility of the same solution indicates a high degree of colloidal dispersion (Höber and Kempner, '08).

Thus we might consider the solution of anilin blue w. s. as containing the particles largely in the colloidal condition or as largely molecularly dispersed.

Regarding the diffusibility of dyes through the dialyzing shell of Schleicher and Schüll, Höber and Chassin ('08) arranged the dyes in the following order according to the grade of their diffusibility:

Though a detailed statement of the dialysis of Biebrich's scarlet is not given, it is certain that this dye was more easily dialyzed than anilin blue. From such considerations we feel justified in placing Biebrich's scarlet between light green F S and anilin blue, in table 5 comparing the

TABLE 5

List of dyes in the order of increasing diffusibility

Diamintgrün, Kongobraun, Azoblau, Hessisch Bordeaux
Salmrot, Palatinschwarz
Säureviolett, *Anilinblau*
Biebricher Scharlach, Croceinschwarz
Patentblau A, Guineagrün, *Lichtgrün*

permeability and the colloid state. If this is done then we will obtain a better parallelism between the permeability of the placenta and the colloid state of the dyes.

Thus we may assume that the colloidal character of dyes is an important factor, not only as regards their passage, at least to a certain extent, through the placenta, but also in respect of their passage through the cell membrane.

To estimate the degree of the colloidal state the solution must be examined for several of its physico-chemical characters; as diffusibility, ultra-microscopic state, viscosity, precipitability by electrolytes, etc. The results of the observation of these characters are generally parallel with each other.

As already mentioned, however, some of these results at times show apparent contradictions. Therefore to investigate the relation between the penetrability of dyes and their colloidal character, comparison must be made with each of these physico-chemical characters. Otherwise

we cannot learn which of these characters has the closest relation to this power.

In my case, however, I collected first the data reported in the literature regarding the physico-chemical character of the dyes to get some orientation for this problem, before I started on my own experiments. By this preliminary study I have learned that the ultra-microscopic character and the diffusibility of dyes, especially the latter, show the most striking parallelism with their ability to pass the placenta. With the present ultra-microscope, however, we are almost unable to estimate the grade of the colloid state of dyes in so detailed a way as by dialysis which enables us to give each dye its numerical grade. Therefore I examined only the diffusibility of the dyes.

In comparing the results of diffusion with the results of the experiments on permeability, I reached an interesting conclusion which will be stated later. Before coming to this statement I wish to discuss briefly the relation between the penetrability and some physico-chemical characters, employing data taken from the literature.

Höber and Kempner ('08) examined the precipitability of the solutions of dyes by electrolytes and observed the relation between the precipitability and the power to pass through the cell membrane in the kidney of the frog. Later Ruhland ('12) also made the same observation, using the plant cell in place of the kidney cell. According to their results, the cell membrane is but little permeable to the easily precipitable dyes, while easily permeable to those hard to precipitate,—though there are a number of exceptions to this rule.

Now let us consider the relation between the precipitability and the ability of dyes to pass through the placenta. Following the results obtained by Höber, Kempner and Ruhland, we may arrange the dyes approximately in the order given in table 6, according to their decreasing precipitability, Congo red being most easily precipitated. In the second column the ability of each dye to pass through the placenta is indicated by the marks + and − which have the same meaning as in serological tables.

Though the number of dyes in this series is small, yet table 6 justifies the conclusion that the permeability of the placenta for dyes has no close relation with their precipitability by electrolytes. Here anilin blue offers the most striking exception.

As the next step we shall consider the surface tension of solutions of the dyes in relation to their ability to pass the placenta. In 1914 Höber made observations on the relation between the relative surface

tension of solutions of dyes and their power to stain the living cell, but failed to find any parallelism between them. He determined the surface tension of the solutions of sixteen basic, and thirty-two acid dyes by means of Traube's stalagmometer. If we employ his stalagmometric results on those dyes which have been used in my experiments on permeability, we obtain the results presented in table 7, in which the

TABLE 6

List of dyes in the order of their decreasing precipitability

	PERMEABILITY OF THE PLACENTA
Congo red	–
Biebrich's scarlet	++
Bordeaux red	+
Fuchsin (acid)	+++
Anilin blue w.	–
Orange GG*	++++
Light green F S.	++

* In my experiment orange G was used instead of orange GG.

TABLE 7

Surface tension and permeability

DYES	NUMBER OF DROPS	PERMEABILITY OF THE PLACENTA
Methylviolet	20.7	++
Dahlia	20.2	+++
Light green F S.	18.1	++
Anilin blue w. s.	18.0	–
Safranin	17.9	++++
Neutral red	17.8	++++
Toluidin blue	17.6	++++
Methylene blue	17.5	++++
Fuchsin (acid)	17.5	+++
Trypan blue	17.5	–
Congo red	17.5	–

permeability of the placenta for each of these dyes is compared with the number of the stalagmometric drops.

This table does not reveal any clear relation between the ability to pass through the placenta and the surface tension. Here again anilin blue is the most notable exception. It is also remarkable that the two dyes—trypan blue and Congo red—to which the placenta is not

permeable—show the same number of the stalagmometric drops as the dyes to which it is readily permeable—methylene blue and fuchsin (acid).

We pass now to the observations on the ultra microscopic character of dyes. The collected data from the literature regarding this physico-chemical character of dyes are given in table 8.

TABLE 8

DYES	ULTRA-MICROSCOPIC CHARACTER	PERMEABILITY OF THE PLACENTA
Bismarck brown	Insoluble (Raehlmann)	+++
Eosin	Insoluble (Michaelis)	+++
Methylene blue	Insoluble (Michaelis)	++++
Toluidin blue	Insoluble (Michaelis) homogeneous (Ruhland)	++++
Fuchsin (acid)	Optically clear (Ostwald) many particles visible (Höber and Kempner)	+++
Methylviolet	Greatest part insoluble (Michaelis) optically clear (Ostwald)	++
Nile blue	Insoluble (Michaelis) colloid (Ostwald)	+
Safranin	Colloid (Ostwald)	++++
Indigo carmine	Many particles visible (Höber and Kempner)	++
Neutral red	Colloid (Ostwald) Greatest part soluble (Michaelis)	++++
Alizarin-SO_3Na	Colloid—polydisperse system (Ostwald)	+
Anilin blue	Completely soluble (Michaelis) Numerous particles visible (Höber and Kempner)	−
Alkali blue	Completely soluble (Raehlmann)	−
Congo red	Highly colloid (Ostwald) Numerous ultra microscopic particles (Höber and Kempner)	−

So far as these ultra microscopic data indicate, Bismarck brown, eosin, methylene blue and toluidin blue are really soluble in water, while the aqueous solutions of anilin blue, alkali blue and Congo red are in a highly colloidal state. The aqueous solutions of acid fuchsin and methylviolet seem to be also true or nearly true solutions. The solutions of the remaining five dyes belong to the class of so-called half-colloids.

If we compare the colloidal condition of these dyes, as shown by their ultra microscopic properties, with their ability to pass through the placenta, we find that the dyes existing largely in the colloidal state do

not get through, while those whose solution is semi-molecular or largely molecular pass through with more or less facility. The grade of dispersion of the dyes is not exactly parallel to the grade of their penetrability. Safranin and neutral red are reported by Ostwald as colloids, and both of them are more effective than acid fuchsin and methylviolet, whose solutions are noted by the same author as optically clear. Regarding safranin, I have been unable exactly to determine whether or not its solution approximates a true solution.

TABLE 9

DYES	BEHAVIOR IN DIALYSIS THROUGH DIALYZING SHELL	PLACENTAL PERMEABILITY
Methylene blue	Quickly dialyzed (Buxton and Teague)	++++
Safranin	Quickly dialyzed (Buxton and Teague)	++++
Toluidin blue	Quickly dialyzed (Ruhland, '08 b)	++++
Fuchsin (acid)	Easily dialyzed (Höber and Kempner)	+++
Eosin	Quickly dialyzed (Buxton and Teague)	+++
Indigo carmine	Easily dialyzed (Höber and Kempner)	++
Methylviolet	Quickly dialyzed (Krafft)	++
Neutral red	Slowly dialyzed (Buxton and Teague) Confirmed by Ruhland, '12	++++
Nile blue	At room temperature not at 37°C. slowly dialyzed (Buxton and Teague) Confirmed by Ruhland, '12	+
Anilin blue w. s.	At room temperature not at 37°C. a little dialyzed (Buxton and Teague) very little dialyzed (Höber and Kempner)	−
Alkali blue	Not dialyzed (Buxton and Teague)	−
Congo red	Not dialyzed (Buxton and Teague) Not dialyzed (Höber and Kempner)	−

It is certain however that the solution of neutral red is nearly half-colloidal, because this dye is said to be for the most part optically soluble in water (Michaelis). As a matter of fact this dye passes the placenta readily. Thus we reach the conclusion that the ultramicroscopic dispersion of the dyes is inversely related to their ability to pass through the placenta, but the degree of dispersion is not exactly parallel to the degree of their penetrability.

Finally we will compare the power of the dyes to pass through the placenta with their diffusibility through the dialyzing shells of Schleicher and Schüll. The collected data from the literature regarding this physico-chemical character of dyes are given in table 9 in which the penetrability of each dye is given in the last column.

In table 9 we find that the diffusibility of dyes gives a better parallelism with their power to pass the placenta than any other of the physico-chemical characters discussed hitherto. Here again, neutral red is slightly aberrant. On the whole, however, we learn from tables 8 and 9 that the permeability of the placenta for dyes is related to the difference in the size of the particles in the solution.

Thus far the permeability of the placenta for dyes has been compared only with the physico-chemical characters of the dyes as reported in the literature. The physico-chemical characters of a dye bearing a given name are not always the same, because most of the dyes in the market are not chemically pure and the grade of the impurity of a dye is not always the same. Even a dye bearing the same name and manufactured by the same company may differ according to sample. In my case, therefore, it was absolutely necessary to test the physico-chemical character of the dyes, using the same samples as were used for testing the permeability of the placenta. By the preliminary study of the literature just presented, we have learned that the ability to pass the placenta is most closely related to the diffusibility of the dyes.

Therefore I investigated the diffusibility of the dyes used in the studies of placental permeability here reported. In this case, however, I tested the diffusibility of the dyes using gelatin, instead of the dialyzing shell of Schleicher and Schüll, because it is possible to control the size of the pores in the gel simply by regulating its concentration, which makes a convenient means for grading of different dyes with regard to the size of their particles in solution. For this test a 5 per cent gelatin solution in hot water was made and poured into a large Petri dish, forming a thin layer of gelatin on the bottom. After cooling, drops of the same size of different dyes were placed on the gelatin, and the spreading of the color observed. By this simple method it is possible to easily estimate the grade of diffusibility of the dyes.

To avoid evaporation the dish was covered with a watch glass containing wet blotting paper on its under side. The dish was kept in the ice box to prevent the growth of bacteria, though precautions against possible contamination had been carefully taken. To make the size of the drops always the same, I made a small ring of copper wire and attached it to a handle. The diameter of the drop made with this ring was about 5.5 mm.

The quantity of the different solutions in a drop was not always the same because of their different viscosities, so that some addition of the solution to the smaller drops was necessary to make them all equal.

Regarding the concentration of the dye, I made a 1 per cent solution of each dye in physiological salt solution, and just before use mixed it with the same amount of blood serum from the albino rat. In cases of Alizarin-SO_3Na, Dahlia, Lithium carmine, Nile blue and Safranin, the serum was mixed with the aqueous solution of each dye.

As is well known, the colloidal state of a solution may be influenced by the addition of another colloid. Consequently, the dispersion of dyes in the circulation will be different from that in physiological salt solution or water. Therefore I mixed the solution of dye with the serum, to make the state of dispersion of the dye in the experimental solution as similar to that in the animal body as possible, and thus to avoid, as far as I could, any factors of error.

At intervals of 24 and 48 hours after the dyes had been placed on the gelatin, the diameter of each colored drop was measured. Under such conditions, the spreading of each color in the 5 per cent gelatin is given in table 10, in which the permeability of the placenta to each dye is compared with the degree to which it had spread.

If we compare the results of the spreading of the dye solution in 5 per cent gelatin with the reports in the literature regarding the diffusibility of the dyes through the dialyzing shell, we shall find a striking similarity between them. Thus our assumption becomes the more certain that the permeability of the placenta for dyes runs parallel with the diffusibility of the dyes and consequently with the size of the particles in the solution. Only one exception is presented in the case of neutral red. While this dye is one of those which penetrate best, its diffusion both through the dialyzer and through the gel is relatively slow. It is also to be noted that all the dyes to which the placenta is not permeable, except alkali blue, show some diffusion in 5 per cent gelatin, though their spreading is slower than that of any of the active dyes. From this fact we may suppose that the placenta as a dialyzer is more efficient than 5 per cent gelatin. In 1912 Ruhland found a surprising parallelism between the permeability of the membrane of the plant cell for dyes and the degree of spreading of those dyes in concentrated gelatin, and explained the process of the penetration of dyes into the cell as a phenomenon of the ultra-filtration working under high pressure. Though Höber and Nast ('13) raised objections to the details of Ruhland's work and his far-reaching interpretation, I was very much interested in his theory. Therefore I made further experiments on the spread of dyes in gelatin of increasing concentration expecting to find a concentration of gelatin which might be considered

to have pores of the same size as has the placenta. With this point in view, I examined first the spreading of dyes in a 10 per cent gelatin. The result of this examination is given in table 11.

In 10 per cent gelatin isamin blue and alkali blue showed no trace of spreading even after 48 hours, but the other five inactive dyes still spread into the gel, though only to a slight degree. In table 11 we

TABLE 10

Spreading of dyes in 5 per cent gelatin

DYES	DIAMETER OF COLOR SPOT IN MILLIMETERS AFTER		PERMEABILITY OF THE PLACENTA
	24 hours	48 hours	
Orange G	31	42	++++
Methylene blue	22	28	++++
Safranin	21	27	++++
Fuchsin (acid)	20	26	+++
Toluidin blue	20	25	++++
Bismarck brown	19	24	+++
Dahlia	19	24	+++
Eosin w. s.	19	24	+++
Light green F S.	19	24	++
Indigo carmine	17	23	++
Neutral red	18	22	++++
Biebrich's scarlet	18	21	++
Methylviolet 6B	16	21	++
Bordeaux red	13	17	+
Alizarin SO_3Na	12	16	+
Nile blue	12	14	+
Anilin blue w. s.	12	13	−
Niagara blue 2B	10	13	−
Trypan blue	10	12	−
Congo red	8	11	−
Lithium carmine	9	11	−
Isamin blue	7	10	−
Alkali blue	5.5	5.5	−

find a remarkable situation for Bordeaux red, alizarin SO_3Na and Nile blue. While methylviolet 6B, standing next above Bordeaux red, spreads to 18 mm. in 48 hours, these three dyes spread only to from 12 to 10 mm. in the same period of time, despite their power of passing through the placenta. This fact indicates that the particles of these three dyes are much larger than those of the other penetrating dyes, and, at the same time, it gives a clear explanation for the low resistance to their

passage through the placenta. We also find that among the seven dyes to which the placenta is impermeable Niagara blue 2B, anilin blue w. s., trypan blue, lithium carmine and Congo red have somewhat smaller particles in their solution than alizarin SO_3Na and Nile blue.

As a dialyzer the placenta seems therefore to have smaller pores than 10 per cent gelatin. The same experiment on 20 per cent gelatin gave the results presented in table 12.

TABLE 11

Spreading of dyes in 10 per cent gelatin

DYES	DIAMETER OF COLOR SPOT IN MILLIMETERS AFTER		PERMEABILITY OF THE PLACENTA
	24 hours	48 hours	
Orange G	22	33	++++
Methylene blue	19	24	++++
Safranin	19	22	++++
Fuchsin (acid)	16	21	+++
Bismarck brown	16	20	+++
Eosin w. s.	15	20	+++
Neutral red	15	19	++++
Toluidin blue	15	19	++++
Light green F S.	15	19	++
Dahlia	14	19	+++
Indigo carmine	14	18	++
Biebrich's scarlet	14	18	++
Methylviolet 6B	13	18	++
Bordeaux red	11	12	+
Alizarin SO_3Na	10	11	+
Nile blue	9	10	+
Niagara blue 2B	7	8	−
Anilin blue w. s.	7	8	−
Trypan blue	7	7	−
Lithium carmine	7	7	−
Congo red	6	7	−
Isamin blue	5.5	5.5	−
Alkali blue	5.5	5.5	−

The results with 20 per cent gelatin apparently show that the dyes, which can penetrate the placenta, can penetrate 20 per cent gelatin, while the dyes which do not penetrate the placenta do not penetrate the same gel.

The intensity of the spreading of the dyes in 20 per cent gelatin is exactly parallel to the intensity of the penetration of the placenta by the

dyes. On the other hand, all the ineffective dyes, except Niagara blue 2B, remained on the gelatin plate in their original size, without any trace of diffusion. Niagara blue 2B formed a very narrow ring around the margin of the original spot at the end of the 24th hour. The ring however did not measurably widen in the following 24 hours. Though

TABLE 12

Spreading of dyes in 20 per cent gelatin

DYES	DIAMETER OF COLOR SPOT IN MILLIMETERS AFTER		PERMEABILITY OF THE PLACENTA
	24 hours	48 hours	
Orange G	16	19	++++
Methylene blue	16	18	++++
Safranin	15	18	++++
Neutral red	13	15	++++
Toluidin blue	13	14	++++
Fuchsin (acid)	13	14	+++
Eosin w. s	12	14	+++
Bismarck brown	11	13	+++
Dahlia	11	13	+++
Light green F S	11	13	++
Biebrich's scarlet	10	13	++
Indigo carmine	10	12	++
Methylviolet 6B	9	12	++
Bordeaux red	8	9	+
Nile blue	8	9	+
Alizarin SO_3Na	7	8	+
Niagara blue 2B	6	6	−
Anilin blue w. s	5.5	5.5	−
Trypan blue	5.5	5.5	−
Lithium carmine	5.5	5.5	−
Congo red	5.5	5.5	−
Isamin blue	5.5	5.5	−
Alkali blue	5.5	5.5	−

I cannot wholly explain this peculiar phenomenon, it seems certain that this dye spreads but slightly, if at all, in 20 per cent gelatin.

Hoping to obtain a more distinct difference between the spreading of the dyes, I made one more experiment using 30 per cent gelatin. The results are given in table 13.

Table 13 shows a striking parallelism between the spreading of dyes in the gel and the permeability of the placenta, as was shown in table 12, but my expectation of a more distinctive difference by the use of 30

per cent gelatin was not realized. Here again, Niagara blue 2B spread from 5.5 to 6 mm. in 24 hours, though it did not measurably widen in the following 24 hours. This was the case also in the 20 per cent gelatin. If this dye really spread in 30 per cent gelatin, then it must show a better spreading in 20 per cent gelatin; but such was not the case. From such

TABLE 13

Spreading of dyes in 30 per cent gelatin

DYES	DIAMETER OF COLOR SPOT IN MILLIMETERS AFTER		PERMEABILITY OF THE PLACENTA
	24 hours	48 hours	
Orange G	15	18	++++
Methylene blue	13	15	++++
Safranin	11	14	++++
Neutral red	10	13	++++
Toluidin blue	10	13	++++
Fuchsin (acid)	10	13	+++
Bismarck brown	10	12	+++
Eosin w. s.	9	12	+++
Dahlia	9	12	+++
Indigo carmine	9	11	++
Biebrich's scarlet	9	11	++
Light green F S	9	11	++
Methylviolet 6B	8	10	++
Bordeaux red	8	9	+
Nile blue	8	9	+
Alizarin SO_3Na	7	8	+
Niagara blue 2B	6	6	−
Anilin blue w. s.	5.5	5.5	−
Trypan blue	5.5	5.5	−
Lithium carmine	5.5	5.5	−
Congo red	5.5	5.5	−
Isamin blue	5.5	5.5	−
Alkali blue	5.5	5.5	−

considerations we feel justified, whatever the cause of this peculiar phenomenon, in saying that this dye is practically indiffusible, in both 20 per cent and 30 per cent gelatin. One may oppose this assumption by the cases of Bordeaux red, alizarin SO_3Na and Nile blue, the spreading of which in 20 per cent gelatin was the same as that in 30 per cent gelatin. Against this however we have the fact that these three dyes spread further and further into the gel in the course of time.

If, for the sake of comparing the observations on the spreading of dyes in the gels of different concentrations, a chart is prepared, it shows that the permeability of the placenta for dyes runs parallel with the spreading of the dyes in the gel, but when the gel is not concentrated, the spreading of dyes is not exactly parallel to their power to pass through the placenta, and in the gel of 20 or more per cent of gelatin, only those dyes which pass the placenta spread in the gel, while the other dyes fail to spread.

From these facts we may assume that the placenta is similar to a concentrated gel-membrane, in so far as its permeability is concerned. That is to say, the permeability of the placenta is to be considered as a phenomenon of ultra filtration in Bechhold's sense ('07–'08), the placenta here corresponding to an ultra-filter. The fact that the basic dyes permeate easily and the acid dyes less easily, or that the benzidin dyes generally do not penetrate, depends in the last instance on the size of particles of the dyes in the blood. So far as my results indicate, the placenta seems to have no mysterious function as a special selector of materials in favor of the fetus, but merely the function of an ultra-filter.

Though my conclusions are based on the results of experiments on the permeability of the placenta for dyestuffs, it seems very probable that the same principle can be extended to explain generally the exchange of various substances between the mother and the fetus, and further systematic investigations along this line, using many other substances, are already planned.[2]

CONCLUSIONS

1. In these experiments the permeability of the placenta of the albino rat and the mouse was studied, using twenty-three dyes; eight basic and fifteen acidic.

2. The placenta was permeable to all of the basic dyes. Among the fifteen acid dyes eight penetrated the placenta, while seven could not be found in the fetus.

3. The permeability of the placenta for dyes is entirely similar in the albino rat and mouse.

[2] In connection with this study 22 protocols, one for each dye, have been prepared. These give a record of the diffusion of the dye in fifty-four localities and organs in both the rat and the mouse. They are now filed among the archives of The Wistar Institute, where they are available for consultation.

4. The power of the dyes to pass through the placenta runs parallel to the colloidal state of their solution in the serum, especially to their ability to spread in a gel of high percentage.

5. So far as its permeability for dyes is concerned—the placenta acts as an ultra-filter.

6. In view of the size of the colloidal particles which can pass the placenta, it is inferred that proteins must be decomposed into their components in order to pass from the mother to the fetus.

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THE LIBERATION OF EPINEPHRIN DURING MUSCULAR EXERCISE[1]

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Received for publication July 14, 1922

Recently we (1) have described experiments in which it was indicated that epinephrin was released during the development of fatigue from muscular exercise. The present paper reports a continuation of those experiments. The technique is similar, with a few exceptions, to that used in the preceding research.

Treadmill. A treadmill with an internal circumference of 5.72 m., which was more than twice the circumference of that used in the previous study, was employed. The tread was 0.5 m. wide, being composed of slats which were spaced and grooved to improve the foothold. The sides of the treadmill were lined internally with transparent celluloid, this being reinforced externally by hardware cloth. Four doors, equally spaced, were provided in the tread. The mill was always driven by hand with a crank connecting with a set of three-to-one gears. One of us made all of the tests because we found that the amount of work which a cat would do, depended very much upon the care exercised in driving the mill. An inexperienced person will often drive the mill so fast that the animal may appear fatigued before he really is. Moreover one must suit the whim of the animal and not his own inclination. This treadmill proved to be more satisfactory than the smaller one because the animal did not have to travel down such a steep incline (cats practically never work on the upgrade).

Records of pupil dilatation. We have made a practice of securing photographic records of both pupils at different stages of muscular exercise. For this purpose it was necessary to use a reflecting camera with a rapid lens. In order that both pupils might be equally illuminated, two banks of lamps (900 watts each) were placed one on either side and about 50 cm. away from the place where the eyes were to be exposed.

[1] This study was aided by a grant from the Research Fund of the American Association for the Advancement of Science.

The banks were set at an angle of about 45° with the eye-camera axis. These banks were turned away from the camera and the lamp filaments obscured by passing the light through white tissue paper. When taking a photograph the cat was held for a few seconds in a marked position. A record made with such a strong constant lighting was independent of variations from the illumination of the room. Moreover the intense illumination contrasted the denervated pupil with the normal pupil more than a weaker light would have done. Although the denervated pupil responds to light like the normal pupil when there is no increase in epinephrin, with such an increase the light reflex only partially affects the denervated pupil. The epinephrin dilatation prevents the light reflex of the denervated pupil so that where one is in doubt as to epinephrin dilatation, the placing of the eyes in stronger light will settle the question. If the denervated pupil remains larger than the control it is fair to assume that there is an increase in epinephrin. When large amounts of epinephrin are being set free and consequently the denervated pupil is greatly dilated, the light reflex has little or no effect.

Denervation of the adrenal. We (1) have previously shown that after cutting the nerves to the adrenal, regenerating fibers find their way back and in the course of a few weeks probably function as before. In order to prevent regeneration, the method of Hartman and Blatz (2) of enclosing the cut nerve in sterile rubber tubing was employed. All connections with the splanchnic nerve were cut and then the semilunar ganglion was pulled into a short piece of rubber tube. The tube was tied tightly at the top and bottom, thus effectually preventing regeneration. Connections with the chain of sympathetic ganglia in the abdomen were usually not disturbed. However, if all of the other nervous connections were destroyed, there was rarely an eye reaction from muscular exercise.

The rubber tube was left in place until the animal was killed. No ill effects from the rubber were apparent. If the animal lived long the rubber tubing became thoroughly encased with connective tissue. Cats were used exclusively in these observations.

The denervated eye reaction as a test for epinephrin in muscular exercise. Experiments additional to those previously reported, confirm our conclusion that dilatation of the denervated pupil during exercise is due to the release of epinephrin. The left adrenal of a cat, from which a superior cervical ganglion had been removed, was denervated in the manner described above. After recovery from the operation, the

denervated pupil gave an excellent dilatation from exercise in the treadmill (fig. 1). The right adrenal was then removed. Upon recovery muscular work in the mill produced no dilatation (fig. 1).

Proof was likewise obtained in a cat from which one adrenal had been removed and the other denervated by cutting the nerve fibers but not preventing their regeneration. Although immediately after recovery from the operation no dilatation could be obtained from exercise, several months afterward dilatation could be obtained from exercise. This reaction gradually improved. However, neither the extent nor the persistence of the dilatation became as great as it does in a cat with one or both normal adrenals. We had no records of this cat preceding the adrenal operation. A little more than eleven months after the first operation, the adrenal was again exposed. Nerve fibers connecting the semilunar ganglion with the adrenal were cut and the ganglion capped with rubber. After recovery, exercise in the treadmill caused a small transient dilatation of the denervated pupil but nothing like that preceding the operation. Some nerve fibers may have escaped section, for on account of adhesions they were more difficult to differentiate than they are in an animal operated upon for the first time.

In all cats with but a single adrenal, and that denervated, we have found that if any dilatation occurs it not only is small in amount but does not persist long after the work stops. In other words, not only the attending secretion but the after-secretion is very much diminished.

Finally we have obtained dilatation of a completely denervated pupil (superior cervical and ciliary ganglia removed) accompanying exercise, thus completely ruling out a possible nervous influence. Five cats have been tested in this way, all giving positive results. (Observations to be published in detail later.)

Latent period for secretion increase. It is impossible to measure exactly the latent period of the epinephrin increase which attends exercise but one can determine the time and work required to produce a noticeable dilatation of the denervated pupil and also the time and work required to reach the maximum dilatation. Mild exercise may show little or no dilatation of the denervated pupil. We led two cats around the room at a moderate pace for 20 minutes, 544 meters being travelled in that time. One cat trotted along willingly, the other held back and had to be pulled along. The first cat showed a small dilatation of the denervated pupil beginning at 51 meters. The second cat showed no dilatation until 500 meters had been passed and then but a slight one. Work in the treadmill for 57 meters (3 minutes) in the first and 143 meters (6 minutes) in the second caused a further increase in the pupil dilatation.

More difficult work slowly performed requires several minutes for the dilatation of the denervated pupil. For example, a cat travelling slowly in the treadmill required 15 minutes for the development of pupil dilatation. However only 80 meters had been passed. A similar dilatation in the same cat, was obtained frequently in 2 minutes when 50 to 100 meters had been travelled. Rapid vigorous work in the treadmill has produced noticeable dilatation in most animals after 1½ to 3 minutes, the distance covered in that time being from 40 to 180 meters. From a study of nine cats having either one or two normal adrenals we conclude that with vigorous exercise the latent period is frequently less than 2 minutes. The conditions which we have just discussed are those in which the animal starts from a long period of rest. If, on the other hand, the cat has been working and has been allowed to rest merely long enough for the denervated pupil to become normal, it seems to require a shorter period of exercise to produce again a noticeable dilatation. It is as if there was facilitation in the nervous paths or some epinephrin was being released above the normal amount, although not sufficient to affect the pupil. In the latter case a small increase would produce the result.

Cats possessing but a single adrenal and that one supplied with regenerated nerve fibers, the nerves having been cut several months before, show a somewhat longer latent period than the normal. This may be due to the incomplete nerve supply.

The magnitude of dilatation. The maximum dilatation reached depends both upon the intensity of the exercise and upon the individual. Hard, vigorous work will produce a somewhat greater effect than will moderate work in the same individual. Certain individuals show a greater response than do others. The experience of Doctor Cannon has been that young black cats show a degree of responsiveness to conditions arousing emotional excitement that is not manifested by other animals.[2] Three young cats which we have studied showed a greater dilatation of the denervated pupil than we usually obtain in adult animals. One of these, a gray tiger cat, was 9 months old and weighed 1.8 kgm. The ages of the other two were unknown. One, a black and white, weighed 1.2 kgm., the other, a black and yellow mixture, weighed 2.2 kgm. Large adults seem to respond to a less extent than do small adults. This may be due to the fact that frequently large animals are clumsier and do not work as well. Small ones appear to be more excitable, are quicker in their movements and likewise appear to release more epineph-

[2] Private communication.

rin. We expect to obtain more evidence on this point before reaching any conclusion.

The time required to reach the maximum dilatation varies considerably and seems to depend upon the muscular effort involved. If the animal works hard continuously the dilatation may increase until it finally becomes maximal. On the other hand if the effort is of only moderate degree the dilatation may reach its high point in less time because that point is lower, that is, the amount of epinephrin per unit of time appears not to become so great finally as in the former case.

After-secretion of epinephrin. One would conclude from the denervated pupil dilatation caused by muscular exercise that the increased outpouring of epinephrin persists for periods of time ranging from a few minutes to a few hours after cessation of the muscular activity. This depends upon both the effort expended in unit of time and upon the length of the work period. On the whole, the greater the dilatation of the pupil, that is, the greater the outpouring of epinephrin, the longer will this after-effect be prolonged. Thus, if an animal has worked hard for a long time, the after-dilatation is apt to persist a long time. A cat in which the dilatation persisted from 3 to 5 minutes after working for 30 minutes (two tests) showed a persistent dilatation for more than 26 minutes following 60 minutes of work. Another cat showed a persistent dilatation for 3 to 6 minutes after 14 to 19 minutes of work, while after 33 minutes of work, the dilatation persisted more than 27 minutes. This is not true for all animals (table 1). One cat showed no greater persistence of the dilatation following 44 minutes work than it did following 14 minutes work, in fact it was a little less, persisting 15 minutes and 13 minutes respectively (see A, table 1).

The three young animals which we have studied showed a longer persistence of the after-effect than do adults, ranging from 26 to more than 280 minutes (see D and E, table 1, also fig. 1). These effects were for working periods no greater than those in adults.

In the later stage of the after-effect the dilatation is usually only slightly above normal (fig.1). It may continue for some time (minutes or in rare instances hours) at this stage but finally the dilatation disappears. There is some evidence that the increased epinephrin does not entirely disappear after the pupil no longer responds, the amount being below that necessary for the pupil reaction. In many instances, if following a period of work, the pupil is allowed to return to normal and the time elapsed after this stage is not too great, it is much easier to obtain a dilatation than before. In one adult, after 108 meters in 5 minutes

and then a rest of 12 minutes, 17 meters of additional work caused a decided difference in the pupils. In young cats an increase in dilatation was produced with great ease once dilatation was started. Frequently picking the cat up by the back of the neck was sufficient to increase the dilatation slightly. This happened in a cat (1.2 kgm.) which had travelled 835 meters in 25 minutes and then had rested 12 minutes. Still another young cat (2.2 kgm.) showed an increased dilatation from merely being replaced in the treadmill after 7 minutes of rest from 595

TABLE 1

Persistence of denervated pupil dilatation accompanying exercise

CAT WEIGHT	DISTANCE TRAVELLED—METERS	MINUTES	PERSISTENCE OF DILATATION IN MINUTES
A 2.54	281	14	15
	1548	44	13
B 2.70	915	29	5
	550	30	3¼
	1302	60	26+
C 2.80	572	14	3
	858	19	6
	1144	25	10
	1372	33	27+
D 1.80	790	30	280+
	1602	63	263+
E 1.21	538	11	26+
	835	24	55
F 2.20	292	3	9
	801	27	170
	384	9	2.5
	572	14	7

meters of travelling. This animal had no dilatation whatever accompanying exercise in the treadmill after denervating the single remaining adrenal. We believe that it can be correctly inferred that an epinephrin increase was the cause of these quick changes in the pupil.

Cats possessing but a single adrenal and that one supplied only by regenerated nerve fibers show a much briefer persistence of the after-effect when it does occur. Cat 101, one year after cutting the nerves to the remaining adrenal, frequently gave but a very transient dilatation which quickly disappeared after stopping the exercise. Once the dilatation did persist 2¼ minutes after travelling 572 meters in 12 minutes. Cat 111 likewise showed transient after-effects 7 months after

cutting nerves to the remaining adrenal. However, the regeneration appeared to be more complete in cat 103, the after-dilatation from exercise being, 11 months after the operation, of about the same length as in the normal adults.

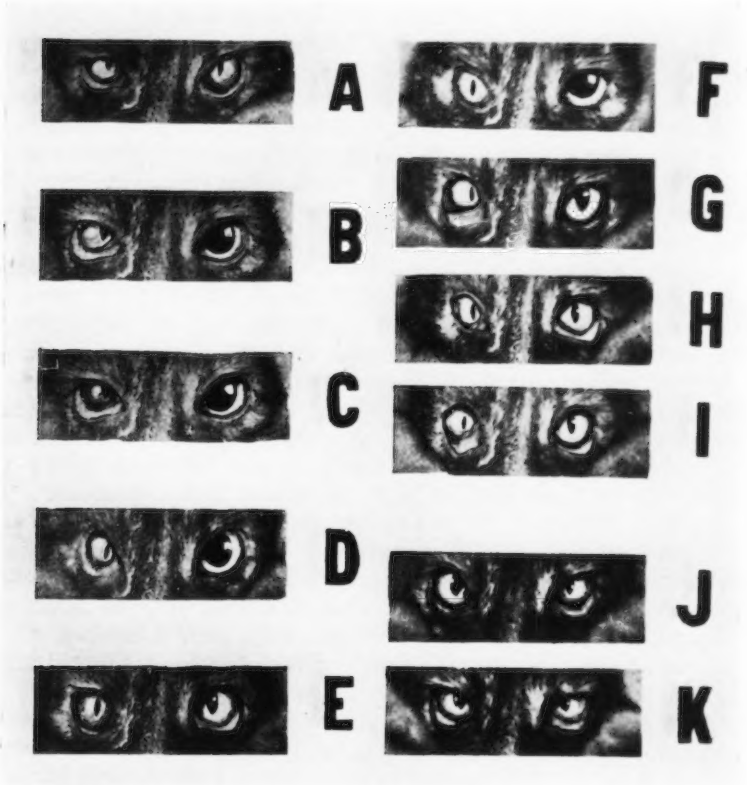

Fig. 1. Response of denervated pupil to exercise, left adrenal denervated. *A*, before. *B*, 292 meters in 3 minutes; 12 seconds after stopping. *C*, 595 meters in 23 minutes; 20 seconds after stopping. *D*, 50 seconds after stopping. *E*, 4 minutes, 17 seconds after stopping. *F*, 810 meters in 42 minutes; 17 seconds after stopping. *G*, 4 minutes, 22 seconds after stopping. *H*, 9 minutes, 33 seconds after stopping. *I*, 15 minutes, 5 seconds after stopping.

Right adrenal removed. *J*, before. *K*, 280 meters in 6 minutes.

We have studied the after-effects in many animals of which the above instances are examples. Without doubt exercise of any consequence is usually followed by an increased output of epinephrin.

The amount of epinephrin released. By the intravenous injection of known amounts of adrenalin chloride it is possible to determine the quantity necessary to produce a certain dilatation of the denervated pupil. In this way if one produces the extent of dilatation which was attained during the exercise it may be assumed with a high degree of probability that roughly a similar amount of epinephrin must have been set free during the muscular activity. Because of the transient nature of the dilatation produced from a few single injections it is, however, not possible to judge the amount of epinephrin with a very great degree of accuracy. But one is at least able to obtain an indication of the amount released physiologically.

Injections were made into one of the veins near the margin of the ear while the cat was wrapped in a sack and the head held firmly by an assistant. These injections were made following the experiment, but some time later than the disappearance of the after-dilatation from exercise, for even after the disappearance of this dilatation the epinephrin output might still be above normal though not enough to reach the pupil threshold. In the latter case it would require less adrenalin to produce the degree of pupil dilatation sought. What we desired to know was the excess of epinephrin released over that of quiet life.

In three cats which we have studied, it required single injections of 0.00130, 0.00165 and 0.00286 mgm. per kilo to produce dilatations of the denervated pupil similar to those caused by from 600 meters to 800 meters of work in the treadmill performed in from 17 to 29 minutes. Because of the methods used in comparing the pupil dilatations, this gives but a rough idea of the amount of epinephrin actually secreted and must in no way be considered quantitative.

Effect of adrenalin injections on muscular activity. We have observed in this and in the preceding study (1) that whenever there is evidence of an increase of epinephrin during exercise an animal can work harder and travel farther than it can without the increase. Therefore the injection of adrenalin should improve the working power of an animal. In our first experiments, we introduced the adrenalin chloride intravenously in order that the exact quantity which reached the circulation might be known. Because of the rapidity of the disappearance of adrenalin so injected we did not expect to produce the improvement in muscular activity which occurs from a more or less prolonged secretion from the

adrenals. A cat with normal adrenals probably would not show the improvement from injections of adrenalin, which might occur in an animal possessing only denervated adrenals, because in the former case there would be a natural increased discharge of epinephrin while in the latter this natural increase would be absent or limited. Therefore we chose, for some experiments, cats possessing a single adrenal and that denervated.

The first cat(weight 3.30 kgm.) had shown a poor response in the treadmill 4 days before adrenalin injections, travelling 866 meters in 42 minutes. It had worked as little as possible throughout the test.[3] Immediately preceding the injection of adrenalin the cat had travelled 103 meters in 5 minutes. It really exerted itself so little in this time that the injection of 0.15 cc. of 1:10,000 adrenalin was made into the ear vein. It then travelled an additional distance of 144 meters in 7 minutes, showing practically no improvement.

A second cat (weight 2.7 kgm.) also possessing a single adrenal, the nerves to which had previously been cut, was injected intravenously with 0.1 cc. of 1:10,000 adrenalin. It was immediately tested in the treadmill. It worked well for 4 minutes going 114 meters. After this point had been reached the cat gradually relaxed its effort. An additional injection of 0.05 cc. of 1:10,000 adrenalin produced no noticeable change. After a total of 740 meters in 48 minutes 0.1 cc. of 1:20,000 adrenalin caused the animal to pace well for 8 minutes (89 meters). The preceding 8 minutes it had travelled only 50 meters and that with very little pacing. At 990 meters (80 minutes) 0.05 cc. of 1:10,000 adrenalin was injected. Again there was improvement, the animal pacing 108 meters in the next 4 minutes. After each spurt due to adrenalin, the animal refused to exert itself. At 1360 meters, 99 minutes 0.1 cc. of 1:10,000 adrenalin was injected intravenously. Another spurt of pacing resulted, the animal travelling 150 meters in 5 minutes. Without a doubt the intravenous injection of adrenalin benefited this animal.

Improvement in the working power was obtained in a third cat from the intravenous injection of adrenalin. This animal possessed a single

[3] It must be explained that distance travelled per unit of time does not necessarily give a true indication of the work performed. The same animal may travel at the same rate in two tests of equal length but may exert himself much more in one test than in the other. In the one test he may pace steadily with a great expenditure of energy while in the other he shirks as much as possible, sliding, clinging and dropping. When the rate does not indicate the true amount of work accomplished it is so stated.

adrenal, the nerves to which had been cut but had later regenerated. This cat when placed in the treadmill worked very well at first. After 45 minutes during which 813 meters had been travelled it became tired. At this stage the injection of 0.2 cc. of 1:10,000 adrenalin enabled it to travel 127 meters in 5 minutes. The rate just preceding the injection was 100 meters in 5 minutes.

In every instance of benefit from intravenous injection, the improvement has been temporary. In order to produce a more lasting effect intramuscular injections were tried. These are absorbed over a long period of time, often a few hours (indicated by the dilatation of the denervated pupil). In all remaining experiments injections were made in the muscles of the back.

Adrenalin, 0.5 cc. of 1:2000 in amount, was injected into cat 145 (weight, 2.72 kgm.) The animal was immediately tested in the treadmill. It refused to work well never pacing more than 3 meters consecutively. After travelling 406 meters in 20 minutes in this unwilling fashion, 1.5 cc. of 1:5,000 adrenalin was injected. When returned to the treadmill, the cat paced 572 meters in 11 minutes without cessation, working vigorously all of the time. The second injection was apparently enough to increase the absorption to the point where it benefited the muscle.

During a test of cat 111, (weight, 2.6 kgm.) although the cat was working hard, "second wind" did not appear (the single remaining adrenal possessed regenerated nerve fibers only). There was likewise no evidence of an increased epinephrin discharge. We thought this a good time to try the injection of adrenalin. The animal was stopped at 572 meters and a total of 3 cc. of 1:10,000 adrenalin injected into different regions of the back. The denervated eye finally became well dilated, indicating good absorption of adrenalin. When returned to the treadmill no improvement was shown during the first 225 meters. Following this a marked improvement was shown for 189 meters, the cat travelling at the rate of 47.2 meters per minute. The previous rate had been 31.8 meters per minute. The animal continued pacing until stopped by convulsions after 128 meters more.

Three weeks later this cat was tested in the treadmill, some epinephrin being secreted at the time (denervated pupil dilated noticeably). It travelled at the rate of 42.6 meters per minute for 12 minutes. Adrenalin, 1.0 cc. of a 1:10,000 solution, was then injected. When replaced in the treadmill the animal paced extremely well, going at the rate of 64 meters per minute for 2 minutes. Following this, the rate decreased

but was maintained for 10 minutes at a higher level than before the adrenalin, being 47 meters per minute.

An experiment with cat 103 (weight, 2.38 kgm.) illustrates the moderate effect from adrenalin injection which may be obtained in a cat which increases its own epinephrin during exercise. This cat possessed a single adrenal with some regenerated nerve fibers (indicated by return of denervated pupil reaction), the nerves having been cut 11 months before. Testing in the treadmill preceding the adrenalin injection showed an increase in the rate of travel as well as a moderate increase in the dilatation of the pupil. During the first 10 minutes it travelled 28.6 meters per minute. During the next 9 minutes it travelled 34.8 meters per minute. This was followed by 7 minutes of work at the rate of 37.0 meters per minute. Adrenalin (2.3 cc. of 1:10,000) was now injected intravenously. During the succeeding 8 minutes the cat travelled at the rate of 39.2 meters per minute showing likewise an improvement in effort (it had been sliding hind legs). An additional injection of 1.0 cc. of 1:100,000 adrenalin was made. There was no further improvement, the cat dropping back to the rate of 37.2 meters per minute for 4 minutes and then to 30.0 meters per minute for 8 minutes, when the test was stopped.

The benefit of intramuscular injection is likewise shown in cat 111 which possessed a single adrenal, the nerves of which had been cut. This cat had been working in the treadmill for 6 minutes at the rate of 30.5 meters per minute before 0.5 cc. of 1:1000 adrenalin was injected. Upon being returned to the treadmill it worked slowly at first, 20 meters per minutes for 6 minutes, and then made a great spurt travelling 172 meters in $3\frac{1}{2}$ minutes.

Finally we studied the effect of intramuscular injections of adrenalin in two cats which appeared to be suffering from adrenal insufficiency. Each cat possessed a single adrenal. This adrenal had been cauterized at two different operations in order to destroy all of the medulla. Later it was discovered that much of the cortex had been destroyed at the same time.

Cat 175 had gradually lost its power to work. Twenty-one days after the first operation (cautery of the medulla of each adrenal through the the mid-line of the abdomen) the animal worked very well. It travelled in this test: 57.1 meters per minute during the first 5 minutes; 47.6 per minute during the next 6 minutes; 49.4 meters per minute in the following 8 minutes and 36.2 meters per minute in the last 6 minutes.

Ten days later the right adrenal was removed. Twenty-eight days after this operation the animal gave the following response in the treadmill: 38.8 meters per minute during the first 5 minutes; 30.4 meters per minute during the next 19 minutes and 17.9 meters per minute during the final 8 minutes.

Eleven days later the remainder of the left adrenal was cauterized the second time through the lumbar route. When tested in the treadmill 10 days after this, the animal appeared very weak. The distance travelled is not comparable with other tests because the animal progressed by sliding and tumbling. During the first 4 minutes the mill was turned at the rate of 21.5 meters per minute, and then for 6 minutes the rate was changed to 25.6 meters per minute. The animal tried but was too weak to accomplish much. A rest of 1½ hours was permitted, during which several doses of 1:5,000 adrenalin were injected into the muscles of the back. When 2.0 cc. had been administered the cat became active, walking about and appearing to feel better. After a total of 12.5 cc. had been injected the animal being placed in the mill became agressive. Instead of sliding it paced and jumped. Even though the treadmill might be going at a slower rate than before the adrenalin the cat was really doing much more work. During this test the cat was able to travel more than twice as far and could have gone farther. It still felt in good condition at the end because it jumped out of the treadmill and stood up, which was entirely different than the finish of the test preceding the adrenalin. The rate of travel was as follows: For the first 2 minutes, 22.5 meters per minute; the next 7 minutes, 18.8 meters per minute; the following 3 minutes, 10.3 meters per minute; this was changed to 26.6 meters per minute during the succeeding 3 minutes and finally a rate of 24.5 meters per minute was maintained for 10 minutes. The cat lived for 7 days after this. It died apparently of adrenal insufficiency.

The results were similar in cat 176. Twenty-six days after cautery of both adrenals the cat worked exceedingly well. During the first 3 minutes it travelled 72.6 meters per minute. For the next 7 minutes the rate was 50.5 meters per minute. Finally the rate was 24.0 meters per minute for 13 minutes. The cat had paced well all of the way and could have gone farther.

Twenty-six days after removal of the right adrenal by the lumbar route the cat again worked well in the treadmill. During the first 3 minutes it travelled at the rate of 63.0 meters per minute. Finally for 13 minutes it travelled at the rate of 39.0 meters per minute.

Nine days after the second cautery of the left adrenal through a lumbar opening, the animal appeared weak. Here again rates of movement of the treadmill are not comparable unless one considers the activity of the animal because it was so weak that although it might try it could do little more than slide. The treadmill was turned at the rate of 20.6 meters per minute for 5 minutes, then at the rate of of 18.2 meters per minute for another 5 minutes and finally for 4 minutes at the rate of 20.2 meters per minute. The cat did not stand when taken out of the treadmill. After the intramuscular injection of 4.2 cc. of 1:10,000 adrenalin it changed from moping to an alert attitude and began to clean itself. After the further injection of 10.5 cc. of 1:5,000 adrenalin, the cat was again placed in the treadmill. It appeared to have lost much of its former weakness. However it resisted so that it would not travel very fast. The test was carried as far as 133 meters, the rate of travel being from 10 to 14 meters per minute. In spite of the lower rate it was actually doing more work than before the adrenalin injections.

It has been demonstrated by these experiments that adrenalin injections can increase the working power of an animal. It should be mentioned that the improvement following adrenalin injections is often accompanied by what appears to be "second wind."

Effect of adrenalin on fatigue convulsions. In previous observations (1) it was found that fatigue convulsions were preceded by an absence or at most a very small dilatation of the denervated pupil. Moreover in the same animal when there was a good dilatation of the denervated pupil convulsions did not develop and the animal travelled farther. In two animals possessing a single adrenal and that denervated (101 and 103) the dilatation was either entirely absent or very small. During that period convulsions were very common. Seven months later convulsions were uncommon in these animals. When they did occur they followed longer periods of work than at the earlier period. In cat 103, the work before these earlier seizures ranged from 476 meters to 850 meters, while 7 months later the work preceding convulsions was about 1400 meters. In this later period there was frequently a fair dilatation of the denervated pupil indicating regeneration of the cut nerve fibers. It proved on later examination that many nerve fibers had regenerated. These observations suggested that epinephrin prevented or delayed the development of convulsions. Therefore we sought to determine the effect of adrenalin injection in animals which were prone to go into convulsions. For our first experiment we chose four cats;

101, 103, 111 and A. All except A had been seized by convulsions during some tests, although convulsions had developed in 111 only once in a number of tests. Intramuscular injections of 0.5 cc. of 1:1,000 adrenalin chloride were made in each of the four animals. These caused a maximal dilatation of the denervated pupil which lasted for a few hours in each instance. All four were tested in the treadmill following the injection. Convulsions appeared at 561 meters, 633 meters and 784 meters respectively in cats 101, 103 and 111. Cat A travelled 1372 meters without any evidence of convulsions. Instead of the convulsions being delayed, they were hastened, for in preceding and following tests, without adrenalin injections, convulsions came much later; e.g., the cat which had convulsions at 784 meters with adrenalin, had travelled 1461 meters before convulsions appeared, 12 days earlier, without adrenalin. Ten days later it travelled 1570 meters without evidence of convulsions. Similar results were obtained in the other two cats.

It might be argued that the doses of adrenalin injected were too large. The amount of adrenalin absorbed per unit of time was certainly above ordinary physiological secretion. So we injected 1.5 cc. of 1:10,000 adrenalin intramuscularly into one of these cats (101) yet convulsions appeared after 1114 meters of work. At another time 1 cc. of 1:10,000 adrenalin was injected. Convulsions developed after 1272 meters had been travelled. It appears from these experiments that epinephrin hastens convulsions in many instances. We thought possibly that the steady diet of liver might have been a factor in convulsions. This is unlikely because the four cats, which we first used in a study of the relation of adrenalin injections to convulsions had eaten nothing but bread and milk for four days previously. The first experiments with adrenalin injections called our attention to the nervous character of the convulsive attacks. Shortly after the injection of adrenalin two of the cats became very much excited. They were so irritable that they fought being held a few seconds for a photograph. Ordinarily they were quite docile when a photograph was being taken. Soon after the onset of convulsions, the cat, lying on its side in the stationary treadmill, would move its feet as if pacing and jumping in the treadmill. It would do this sometimes as long as 20 seconds. Convulsions seemed to develop most easily in cats which were easily excitable. Clumsy, lethargic animals in our experience are not subject to them.

A few minutes after a convulsive attack, the animal appears normal but sometimes sleepy as it does after tiring exercise. There never have been ill effects resulting from convulsions.

Epinephrin release during "warming up" and "second wind." We noticed previously (1) that, when cats appeared to gain "second wind" while working in the treadmill, this was attended by an increased output of epinephrin (eye reaction). We have confirmed this with later observations. During this stage there is an increased output of work as the following examples show.

A cat, upon being exercised in the treadmill, travelled at the rate of 22.6 meters per minute for 6 minutes, the denervated pupil dilatation beginning to appear. During the next 11 minutes, the animal being slightly fatigued, travelled at a lower rate, viz., 21.2 meters per minute. The epinephrin output increased more and more. At the end of the 11-minute period the cat appeared to have gained "second wind" beginning to pace unusually fast and vigorously so that during the final 8 minutes it was going at the rate of 35 meters per minute. The denervated pupil was dilated maximally at the end. Several days later the same cat was tested over a longer distance. The denervated pupil gradually increased throughout the test until it was finally almost maximal. As before, the rate of travel after a preliminary increase in the first few minutes decreased and then increased again. The following were the rates per minute for succeeding intervals there being no rest periods between: 3 minutes, 29 meters; 7 minutes, 37.6 meters; 10 minutes, 34.0 meters; 15 minutes, 28.6 meters; 14 minutes, 25.0 meters; 11 minutes, 30 meters; 23 minutes, 34.8 meters; 17 minutes, 24.8 meters; 20 minutes, 24.0 meters; 11 minutes, 10.0 meters. A later test gave similar results.

Another animal, which was able to work harder than cats usually do, showed the second increase in rate of travel, the epinephrin output increasing throughout the experiment. The variations in rate of travel per minute were as follows: 1 minute, 74 meters; 2 minutes, 60 meters; 4 minutes, 35.7 meters; 7 minutes, 32.7 meters; 5 minutes, 57.2 meters; 6 minutes, 47.7 meters.

If a cat is exercised with intervals of rest, after each rest period it does not work so well at first, but after "warming up" it shows decided improvement. Accompanying this there is an increased output of epinephrin (eye reaction). The following serves as an illustration: A cat travelled in the treadmill for 5 minutes at the rate of 35.4 meters per minute, going faster toward the close than at first. It was allowed to rest 6 minutes during which the denervated pupil had decreased to nearly normal size. Upon being returned to the treadmill it travelled 23.6 meters per minute for 9 minutes. The rate was increased to 29.6 meters for the next 5 minutes, the denervated pupil dilating at the same

time. A rest of 10 minutes was permitted. The epinephrin output was still much above normal at the start of the next work period. When placed in the treadmill the easy breathing as the exercised progressed gave evidence of "second wind". The cat showed a very decided improvement, averaging 43.0 meters per minute for the next 10 minutes.

Thus we see that "warming up" and "second wind" are attended by an increase in the output of epinephrin.

Discussion. The work of Carnot and Josserand (3) indicates that epinephrin is actually used up in fatigued muscles. The injection of 0.05 mgm. of adrenalin per kilo body weight into the femoral artery of a fatigued limb produced about one-sixth of the rise in blood pressure which was obtained by a similar injection into the femoral artery in the opposite leg which was at rest. They inferred that fatigue products destroyed the epinephrin. Our observation that the increased epinephrin output persists some time after the cessation of the exercise fits nicely with this if the function of epinephrin is to remove fatigue effects. Many workers (4) have shown that adrenalin increases the working power of a fatigued muscle.

The "warming up" process which is used as a preliminary to athletic contests undoubtly starts the increased secretion of epinephrin. This might account at least in part for the better results obtained after "warming up" than before. Likewise the improvement accompanying "second wind" may be due partly to an increase in the epinephrin output. It was Cannon (5) who first suggested that "second wind" consists of a setting in operation of the reinforcing mechanism which is accompanied by adrenal secretion.

Injections of adrenalin into intact animals appear to be most beneficial in exercise if given intramuscularly. When injected subcutaneously it is absorbed too slowly, while injected intravenously it is quickly destroyed. Intramuscular injections are absorbed in large enough amounts over a prolonged period to simulate more nearly the increased output during excercise and therefore appear to be most beneficial.

We have used fifty cats in the study of the relation of the adrenals to exercise. All of them have been kept for at least a few weeks. Many have been studied for several months. Two have been studied for 16 months.

Although there is wide variation in the behavior of individuals, we have tried to present typical examples.

SUMMARY

Proof is given that the increased dilatation of a denervated pupil (superior cervical ganglion removed) during exercise is due to epinephrin. The latent period for the beginning of the increase in epinephrin during vigorous exercise must be less than 1½ to 3 minutes. The increase appears much later in mild exercise.

The maximum output of epinephrin reached depends upon the intensity and duration of the exercise. After the exercise ceases the increased output of epinephrin persists usually for a few minutes and after vigorous exercise of long duration sometimes for a few hours. The increase is gradually diminished until it disappears altogether. There is considerable individual variation in the after-secretion.

Adrenalin injections usually, although not invariably, improve the working power of the individual. These improvements resemble the "second wind" which is observed in normal cats accompanying dilatation of the denervated pupil. The injection of adrenalin hastens the onset of fatigue convulsions.

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FURTHER OBSERVATIONS ON THE EFFECTS OF THE SUBCUTANEOUS INJECTION OF SPLENIC EXTRACT

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Received for publication July 14, 1922

The experiments to be reported in this communication are divided into two series; one extending over a period of 106 days, the other more intensive, confirmatory of the first and showing additional features of interest. All the experiments were performed on rabbits. In the first group there were five rabbits subjected to subcutaneous injections of extract of the spleen and two controls. The injections were made every day, the appropriate dose of splenic material being dissolved in normal saline solution, 0.7 per cent. At the same time the control rabbits were given an injection of a corresponding quantity of normal saline solution. The splenic substance used was prepared from the desiccated spleen furnished by Armour & Company. This material was rendered practically protein-free, as described previously (1). The initial dose was 10 mgm. per kilogram of body weight. Later the dose was increased to 20 mgm. per kilo and finally to 40 mgm.

The animals were weighed at intervals throughout the course of the experiment. The weight curves are parallel for those receiving splenic extract and for the controls. Both red and white corpuscles were counted. The white corpuscles showed no change other than the usual variations. The average of the last leucocyte counts was almost exactly the same as before the injections were begun. The red corpuscles, on the other hand, showed an increase in both the spleen rabbits and in the controls. This result is shown graphically in figure 1.

In those rabbits which received the spleen extract there was a marked rise in the erythrocyte count the day following the initial injection. This confirms the result reported by Krumbhaar and Musser (2). Also after each increase in the dose the number of red corpuscles in the circulation showed a decided increase. This initial rise was followed immediately by a decrease in the count, which in turn was succeeded

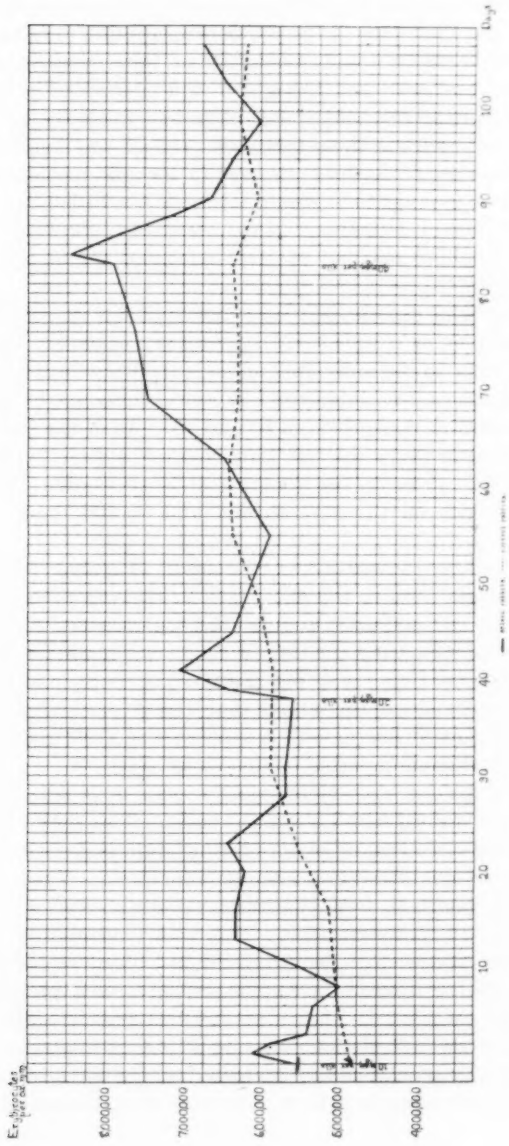
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Fig. 1

by a second rise within 14 days. The second rise was always better sustained than the first. So far as the control rabbits are concerned, the increased erythrocyte count was what we have always found to occur in rabbits purchased and brought to the laboratory where they receive better and more abundant food. The increase in the number of circulating red corpuscles in these rabbits is gradual and does not show either the sudden rises or declines observed in the spleen rabbits.

We believe these findings to be confirmatory of conclusions previously reached as to increased destruction of red corpuscles when this splenic preparation is administered and of their increased production under such circumstances (3), (4).

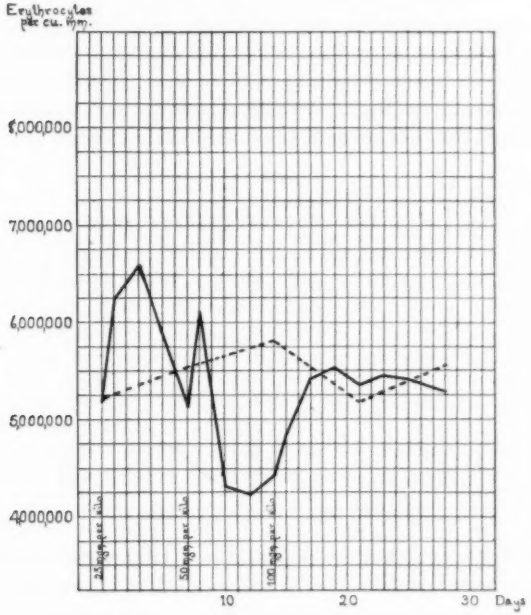
The second series of experiments lasted 28 days and six rabbits were employed; four receiving splenic substance and two controls. As it will be necessary to refer to the individual animals their numbers are given: those to whom spleen was administered were numbered 5, 165, 166 and 167; those used as controls were 12 and 20. As in the first series, injections were made every day of "protein-free" splenic extract but the initial dose was 25 mgm. per kilogram of body weight. This was increased to 50 mgm. on the seventh day and to 100 mgm. on the fourteenth.

Both sets of rabbits showed a loss of weight. The loss was greater in the spleen rabbits than in the controls. In the experiments of the present series the red corpuscle counts were made every second day and at the time of increasing the dose a count was made on that day and also on the succeeding day. The erythrocyte count in the control rabbits showed no change; in the rabbits that were given substance of the spleen the count showed fluctuations comparable to those noted in the first series of experiments reported in this paper, figure 2.

At the time of making each blood count smears were made. These were subsequently stained with Leishman's stain. The smears from the control rabbits showed a normal picture throughout, while in those from the other group several interesting features were found. After the first week the stained specimens from the spleen rabbits showed wide variations in the size of the red corpuscles. This was confirmatory of what had already been observed in the fresh, diluted blood at the time of making the counts. Corpuscles of various sizes became more noticeable as the experiment progressed. Also after the first week irregularities of staining similar to the reticulation of young cells were evident. In the circulation of all four rabbits on the fifteenth day, the day after the dose was increased to 100 mgm. per kilogram, nucleated red corpuscles were

present. Their number increased steadily and at the termination of the experiment averaged 13 per cent.

Special staining was carried out for the purpose of determining the presence of reticulated cells. A saturated solution of brilliant green in 0.7 per cent sodium chloride solution was first prepared and carefully filtered. Five cubic centimeters of this were mixed with 5 cc. of 0.7 per cent sodium chloride solution to which had been added 2 cc. of 2



———Spleen rabbits .....Control rabbits

Fig. 2

per cent sodium oxalate solution. A good sized drop of blood was drawn into the pipette used for diluting blood when counting red corpuscles and the stain used to fill the diluting chamber. Blood and stain were thoroughly mixed, allowed to stand 10 minutes and centrifugalized. The cells were then spread on a microscope slide as in making a blood smear. The ratio of reticulated cells per one hundred erythrocytes was noted. The average in the blood of the controls was 1.5 per cent and in the spleen rabbits 40.0 per cent.

The resistance of the red blood corpuscles was also determined. This was accomplished by using solutions of chemically pure sodium chloride in distilled water, ranging in strength from 0.550 per cent to 0.150 per cent, the decrease each time being by 0.025 per cent. One cubic centimeter of each solution was placed in a small test tube, a drop of blood added, the tube inverted to mix the blood and salt solution and the mixture allowed to stand for 24 hours. Then without disturbing the tubes they were examined and readings made of the tubes showing no hemolysis, partial hemolysis and complete hemolysis. A similar test was made on each rabbit and the results for all six are shown graphically in table 1. The lower percentages of sodium chloride, from 0.150 to 0.225, are omitted from the table as hemolysis was complete in all. This test shows that the resistance of the red blood corpuscles present in the

TABLE 1

ANIMAL	PERCENTAGE STRENGTH OF SODIUM CHLORIDE SOLUTIONS											
	0.550	0.525	0.500	0.475	0.450	0.425	0.400	0.375	0.350	0.325	0.300	0.275	0.250
Control													
12	0	0	+	+	+	+	+	+	+	−	−	−	−
20	0	+	+	+	+	+	+	−	−	−	−	−	−
Spleen													
5	0	+	+	+	+	+	+	+	+	+	+	+	−
165	+	+	+	+	+	+	+	+	+	+	+	−	−
166	+	+	+	+	+	+	+	+	+	+	−	−	−
167	+	+	+	+	+	+	+	+	+	+	+	+	−

0, no hemolysis; +, partial hemolysis; −, complete hemolysis.

circulation of the rabbits that had been receiving splenic extract varied more than is normally the case (5) and more than that of the corpuscles in the control animals of our experiment. Apparently some of the erythrocytes underwent hemolysis more readily and some less readily than normal.

CONCLUSIONS

1. Previous conclusions with regard to the action of "protein-free" splenic extract on the number of red corpuscles in the circulating blood of the rabbit are confirmed.

2. Subcutaneous administration of "protein-free" splenic extract causes:

a. Reticulated cells to occur in the circulating blood in a proportion much greater than normal.

b. The appearance of nucleated red corpuscles in the rabbit's circulation.

c. The resistance of the circulating red corpuscles to extend over a wider range than normal.

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STUDIES IN ABSORPTION FROM SEROUS CAVITIES

IV. On the Passage of Blood Cells and Granules of Different Sizes through the Walls of the Lymphatics in the Diaphragm

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Received for publication July 15, 1922

Previous to the work of von Recklinghausen, the lymphatic system was thought to be a series of channels hollowed out in the general tissues and bordered by non-specific parenchymal cells, more or less loosely bound together. Obviously, with such a conception of the lymphatics, there was no necessity of any theory to explain the appearance within the lymphatic vessels of substances introduced into the tissues. Von Recklinghausen in 1862 (7) demonstrated by means of silver nitrate that the lymphatic vessels are lined by flattened cells which are fitted together in a mosaic pattern. These endothelial cells he considered as specific lining cells, but he did not consider that they formed a continuous membrane. He described the lymphatic vessels as connected with the tissue-spaces by lymph-canaliculi opening into the lumen of the vessels by means of stomata in the vessel walls. These canaliculi he believed served for the passage of material from the tissue-spaces into the lymphatic vessels. This theory was alternately supported and attacked by numerous workers during the thirty years following it first presentation. A very careful and critical review of this work has been given by MacCallum (5).

MacCallum's own work (5), (6) seems to have been the final statement of the view, already becoming established, that there are no preformed openings in the walls of lymphatic vessels, but that the endothelial lining is everywhere continuous; and that absorption must follow some other route. He suggests (6) that the greater part of such foreign material is phagocytized by free cells, with amoeboid capabilities, which then make their way into the vessels with their load of particles.

In his study of the absorption of granular material from the peritoneal cavity he could find no evidence of preformed openings between the mesothelial lining cells, or any channels connnecting the general peritoneal cavity with the lymphatics of the diaphragm. After much careful experimentation he concludes that the passage of granules from the peritoneal cavity into the lymphatics of the diaphragm is effected in three ways: first, the peritoneal lining cells actively phagocytize particles of the material introduced and transfer them to the adjacent lymphatics; second, other particles are forced between the borders of the lining cells by the force of respiration and thus pass between the cells rather than through the cytoplasm; finally, the largest part of the absorption of particulate matter he refers to the intermediation of leucocytes.

In an attempt to analyze the relative importance of the three factors suggested by MacCallum it was thought that a study of the rates of the absorption of particles of different sizes might be of value. And while the results have not permitted the absolute determination of the relative importance of each factor, they certainly indicate that the first is the most important with regard to the lining cells of the peritoneum, and even more so in the passage of granules through the lymphatic endothelium.

For the experiments reported here young adult cats were selected, as uniform in size and weight as possible. Thirty cubic centimeters of a mixture of erthrocytes (obtained from a chicken and carefully washed), large unfiltered carmine granules, and very fine lampblack granules, made up in isotonic sodium chloride solution, were injected into the peritoneal cavity of each animal. The lampblack was filtered several times to remove all of the larger masses. The animals were killed at intervals varying from 1 to 30 minutes; and the diaphragm and anterior mediastinal glands were examined histologically. It was found that after 3 minutes' exposure all three types of material had reached the anterior mediastinal glands, and at this time practically none of the granules were phagocytized, but all were free in the sinuses. In experiments in which the exposure was from 5 to 10 minutes, there was a very small amount of phagocytosis to be seen in the sinuses of the mediastinal glands. This phagocytosis had evidently taken place *in situ*, because in the same experiments there were no cells with granular inclusions to be found in the lymphatic lacunae or vessels of the diaphragm itself. In these localities all the granules were free in small clumps and masses. With increasing increments of time more and more granules

were found within both free and fixed cells in the lymph-glands, until at 30 minutes the majority of the carmine and ink granules had been phagocytized; but the greater part of the red blood cells still remained free in the sinuses of the lymph-glands. In the lacunae of the diaphragm only a few phagocytic cells were discovered at this time. It was thus evident that during the first 30 minutes of exposure very little of the particulate matter introduced into the peritoneal cavity was removed by free phagocytic cells.

Careful examination of the peritoneal side of the diaphragm showed the lacunae in large measure filled with cells, ink and carmine and both the mesothelial surface-cells and the endothelial cells of the lymphatics were found to contain numerous particles of ink and carmine. A very careful search through large numbers of sections failed to reveal a single leucocyte with any of the foreign matter passing through or between the surface-cells or through the lymphatic endothelium. And also at no place was there any evidence of granules or red blood cells passing between the mesothelial or endothelial cells. All along the surface of the diaphragmatic mesothelium there were large numbers of granules and some red blood cells tightly adherent to the peritoneal surface of the lining cells, usually so placed that the flattened axes of the red blood cells were parallel to the flattened axes of the surface-cells. As in MacCallum's experiments, large numbers of granules could always be seen both in surface-cells and lymphatic endothelium. In the former they were indiscriminately placed, while in the latter they usually occupied an irregular perinuclear zone.

The most significant finding in these experiments was the occasional presence of a red blood cell definitely within the cytoplasm of an endothelial cell. Such a group of findings certainly indicates that many of the fine granules and at least part of the red blood cells are passing through the cytoplasm of the lymphatic cells while no evidence was obtained that in the entirely normal animal any of the particulate material passed between the cells.

Krogh and Harrop (4) in attempting to explain the rapid stasis, accompanied by a packing of the capillaries with red blood cells, which follows the use of local anesthetics, postulated a sufficient separation of the endothelial cells to permit the passage of serum, but not blood corpuscles, through the small openings so produced. In order to test this question they injected India ink into the veins of the tongue and then dilated the capillaries by applying a drop of urethane. No ink was seen to leave the vessels. When the same experiment was per-

formed with vital red, the red was seen to pass suddenly out of the capillary and a zone of red was seen outside the vessel.

These experiments do not prove that the vital red passed through openings between the cells, but might equally as well indicate some change in the permeability of the cytoplasm brought about as a result of the dilatation. These experiments are of special interest in regard to the findings reported here because, if the passage of the vital red particles takes place through physical openings temporarily produced between the cells, we then have an approximate measurement of the size of these openings. And if any of the absorption into the diaphragmatic lymphatics takes place in a similar manner, then the ability of the lymphatic endothelium to separate and form intercellular spaces is many hundred times greater than that of the blood-vascular endothelium. Such an hypothesis does not seem likely, and in consequence it seems at present more justifiable to accept the idea of absorption taking place through the cellular cytoplasm.

The experiments of E. R. and E. L. Clark on the reactions of various types of cells in the tadpole's tail have been very illuminating with regard to the activities of the lymphatics. E. R. Clark in 1909 (1), while watching the growing vessels of the tadpole, found that a few red blood cells would occasionally escape from adjacent blood vessels into the tissue-spaces. In a short time the growing tip of a lymphatic vessel would approach these cells, they would be taken within the cytoplasm of the solid tip, and gradually transferred along the sprout to the vessel-lumen. It seems that in this mechanism there is a very definite and specific arrangement for the return to the circulation of erythrocytes which have escaped into the tissue-spaces; and this reaction of lymphatic endothelium was entirely specific and never extended to blood-vascular endothelium. Later Clark and Clark (2) found that when droplets of various fats were introduced into the tail of the tadpole the endothelium of the lymphatics grew toward the fats. They also found that leucocytes phagocytized the fat and after wandering up against the wall of a lymphatic would remain there until the droplets of fat had disappeared from the bodies of the leucocytes and had appeared within the lumen of the lymphatic vessel. Still later Clark and Clark (3) found that particles of ink injected into the tissue-spaces of the tadpole were taken up principally by leucocytes and wandering cells but also to a smaller extent by connective tissue-cells and lymphatic endothelium, these two latter types of cells did not wander toward the ink-granules, but only took up those which were in their immediate neighborhood.

These observations are of considerable interest because they demonstrate the high phagocytic capability of the lymphatic endothelial cells, and assist us in understanding the enormous use of this power which must take place in the removal of large amounts of particulate matter from the peritoneal cavity. There is need of much further work on the permeability of both blood-vascular and lymphatic endothelium, and the work of Krogh and Harrop, in particular, seems to me to be pointing in the right direction.

The results of my own experiments indicate that most, if not all, of the transfer of granular material from the peritoneal cavity into the diaphragmatic lymphatics, during the first 30 minutes, takes place by means of a type of phagocytosis. That later on leucocytes do bring in loads of granules is undoubted, but it seems very likely that the large inflow of free granules continues so long as any remain free in the peritoneal cavity.

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STUDIES IN ABSORPTION FROM SEROUS CAVITIES

V. The Absorption of Particulate Matter from the Peritoneal Cavity of the Fetus

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Received for publication July 15, 1922

The theory of preformed openings between the peritoneal cavity and the diaphragmatic lymphatics which was initiated by the work of von Recklinghausen has not yet been eliminated from the textbooks on histology and physiology, despite the very clear evidence which has been adduced against this theory by Muscatello (9), Kolossow (6), Bizzozero and Salvioli (1), MacCallum (7) and others. The work of these authors has established beyond question that the lining cells of the peritoneal membranes form a physically complete layer and that there are no preformed openings into the lymphatics; the so-called stigmata and stomata. Much work was also done toward determining the mechanism by which particles could enter the lymphatics, after it became clear that there are no stomata connecting the peritoneal cavity with the diaphragmatic lymphatics.

The pathways of absorption from the peritoneal cavity have been very certainly delineated in the course of the last twenty years' work, and it has finally become clear that particulate matter for the most part enters the diaphragmatic lymphatics and passes thence by the anterior mediastinal trunks to reach the anterior mediastinal glands. From there the drainage may be either to the right or left thoracic trunks. A smaller amount passes posteriorly and enters the thoracic duct in the lower chest, that is, follows the usual course of the dorsal efferent trunks of the diaphragmatic plexus. That the other lymphatic plexuses of the peritoneum have not been found to take any appreciable part in this removal of material from the cavity has been shown very clearly and well by a recent report of Bolton's (2). The question of a classification of substances into two groups,—those substances which will pass into the blood vessels and those whose physical or chemical properties

prohibit their entrance into the vascular circulation, but which still permit their penetrating the walls of the diaphragmatic lymphatics,—has not yet been adequately studied. The majority of investigators have concluded that only true solutions of salts can gain entrance to the blood vessels, while Shipley and Cunningham (10), in a series of experiments, demonstrated that numerous colloids and even very fine India ink granules could pass directly into the blood vessels of the omentum.

Admitting, however, the diaphragm to be the most important area for the absorption of particulate matter two questions are of principal interest: in the first place, what are the forces which bring about the passage of the material from the peritoneal cavity, and what route do these particles take in their passage? The most generally accepted opinions regarding the first of these, are that the free phagocytic cells of the peritoneal cavity take up large quantities of the particulate matter and make their way by active amoeboid movement through the mesothelial layer of the diaphragm and the endothelial lining of the lymphatics into the lymph vessels of the diaphragm. Another well-recognized factor is the respiratory movement of the diaphragm and the consequent pressure applied upon the materials free in the peritoneal cavity. MacCallum (7) and Bolton (2) have shown that the movements caused by artificial respiration were sufficient to force materials from the peritoneal cavity into the lymphatics of animals which had been dead for several hours. General intra-abdominal pressure has often been assumed to be an additional factor; it is perhaps better to consider it the basic factor which is being continually modified by the intermittent pressure produced by the movements of respiration.

The actual mechanism involved in the passage of the particles from the peritoneal cavity to the lumen of the lymphatic has been explained in two general ways. In the first place, that the particles pass through intercellular spaces which are formed by the force of the respiratory movements; that is, the particles are forced between the lining cells by the movement of the diaphragm, the cells being only in apposition and not fused together. Again it has been suggested that some or all of the materials absorbed may pass through the cytoplasm of the cells and not between them, and thus the absorption becomes dependent upon other factors than simply being forced or sucked through a space formed "for the occasion." These theories have not been analyzed so sharply as given here, most workers, as MacCallum for example, finding both processes involved in the absorption of particles. A few observers have considered only the mechanical forces and the separation of the cells as

the important factors; this is especially true of the recent work of Bolton (2).

In attempting to analyze the mechanical factors involved in the forcing of materials into the lymphatics, the histological controls were difficult to interpret. In a further attempt to analyze these factors resort was made to experiments on the fetus, it being hoped that the conditions there would be sufficiently simple to permit of a more exact and careful analysis of the location of particles arrested by fixation in their passage through the peritoneal membranes. It was further hoped that perhaps some evidence could be obtained regarding the mechanism of the passage of the particles through the walls of the lymphatics, a phase of the problem which has been insufficiently discussed in the past. It is strange how little is known about this part of the general problem and how consistently it has been avoided in all discussions of absorption from the peritoneal cavity. The meager work which has been done on the passage of particulate materials through the walls of lymphatics has been reviewed elsewhere (5), in connection with the report of some of my own experiments.

The fetuses used in this series of experiments were between 50 and 120 mm. in length. Cats were found to give the most satisfactory results and were used exclusively. The pregnant cat was anesthetized and tied on her back, an abdominal incision was made and the pregnant uterus delivered. The fetuses rotated until the abdominal region was held between thumb and finger when the needle of a hypodermic was plunged through both the uterine and abdominal walls with a single stroke. About 1 cc. of a suspension of lampblack or India ink was injected into the peritoneal cavity and the needle removed. The uterus was returned to the abdominal cavity of the mother and the incision closed. In some cases all the fetuses were left until the end of the experiment, in others they were removed at intervals. The method of removal was to incise the uterus and deliver the fetus, the abdominal cavity of the fetus was then immediately incised in order to prevent the few spasmodic attempts at respiration from having any effect on the abdominal contents. The head was severed from the body as quickly as possible, the fetus eviscerated, the diaphragm rinsed gently with warm Locke solution, the chest opened, and the entire thorax plunged into the fixative. Dissections were made after fixation had been allowed to progress for several hours. Smears were occasionally made from the peritoneal contents and fixed in methyl alcohol for study of cell-types and amount of phagocytosis. The tissues were generally fixed in formalin, Bouin's or bichloride acetic.

In examining the material from these experiments it soon became evident that absorption via the diaphragmatic lymphatics did not take place to any marked extent in the earlier stages. In the older fetuses the amount and rapidity of absorption increased consistently until in those at term there was a very appreciable amount, though the time required for absorption was considerably greater than in the adult and new-born. It has not been established exactly when the fetus begins to make respiratory movements, but there is considerable evidence that it is quite early, at least as early as the middle of gestation. Cat fetuses delivered from the uterus but kept connected to the umbilical cord showed some spasmodic respiratory efforts at stages from 75 mm. to term, usually about 130 mm. No specimens between 50 mm. and 75 mm. have been obtained and the ones at 50 mm. did not show any respiratory effort.

My experiments on the fetus may be divided into three groups, according to the size of the fetuses. The first groups included those under 50 mm. in length, in which no respiratory movements were observed and no granular material had succeeded in reaching the anterior mediastinal nodes. The second group included all fetuses between 75 mm. and 90 mm. in length, and in these a small amount of the granular material had reached the anterior mediastinal nodes after an exposure of 1 hour. In fetuses of this size some spasmodic efforts at respiratory movements were observed. The third group of fetuses included those from 90 mm. to term. In these there were very definite respiratory movements and a considerable absorption had taken place at the end of 1 hour. Shorter exposures demonstrated that granules of the injected material began to reach the anterior mediastinal nodes at 30 minutes.

In the fetuses of approximately 50 mm. length there was a fairly even distribution of the ink throughout the peritoneal cavity with a definitely greater amount on the diaphragm, but no evidence was obtained of any ink in the anterior mediastinal nodes even after an exposure of 4 hours, which was the longest carried out. The diaphragmatic lining cells were found to have a considerable amount of ink attached to their surfaces and there was some evidence of phagocytosis but not very much. There was no histological evidence of the passage of any particles between the cells and there were no particles in the lymphatic vessels. The omentum, mesentery and pelvic lining cells showed considerable amounts of adherent particles but only occasional evidences of phagocytosis. No adherent particles were found on the other visceral surfaces.

In the fetuses of 75 to 85 mm. a very different reaction was observed. After exposures of 30 minutes no granules of ink were found in the anterior mediastinal nodes but after exposure of 1 hour these nodes showed a very small amount of the granules collected in the peripheral sinuses. Histological study of the diaphragm of these fetuses revealed a very interesting phenomenon. The layer of lining cells was very evenly coated with black granules of ink which were quite evidently tightly adherent to the surface of the cells since brisk washing of the diaphragm with salt solution did not dislodge them. In cells so very thin as the adult mesothelial cells the determination of the position of the particles, as on the surface or within the cytoplasm of the cells, is extremely difficult if not wholly impossible. But in the fetus at the stage cited above, the surface-cells of the peritoneum are much thicker, have less widely distributed cytoplasm, and many more nuclei in a given area. It was, therefore, much easier to determine whether the granules were merely adherent or were actually within the cellular cytoplasm. The distribution in these fetuses was extremely regular covering the cell from border to border and including the nucleus as well as the remainder of the cell-surface. No accumulations have ever been seen at cell-junctions. In exposures of 1 hour's duration the ink granules were still scattered over the surface, but were also to be found within the cytoplasm of the cells, rather evenly distributed, but showing some tendency to accumulation in the region of the cell about midway between the nucleus and the periphery; the borders of the cells could only be estimated by the relative position of the neighboring nuclei. A similar appearance was very clearly seen in the walls of the lymphatics where the endothelial lining cells clearly contained black granules and usually most of them were near the nucleus.

In fetuses which were near term, about 100 to 125 mm. in length, the time required for the first grains of black to reach the anterior mediastinal nodes was about 20 to 30 minutes. In one hour's time quite a large amount of ink had passed to the anterior mediastinal glands; the sinuses were entirely filled and some of the granules which had previously been free in the sinuses were beginning to be taken up by certain of the cells of the gland: endothelial cells, reticular cells and histiocytes. Histologically the diaphragm at this stage presented the same appearance as in the stage described above, except that the cells were more flattened and the nuclei further apart. Here it was more difficult to rule out the participation of the intercellular spaces in the active absorption, but in no case did there seem to be anything com-

parable to the concentration which MacCallum found at these regions in the adult.

As a control for these experiments on fetuses, a few were carried out on kittens a few days old. Here it is evident the conditions were almost the same as far as the tissues were concerned, and different only in the increased respiratory activity of the diaphragm. In these animals a suspension of India ink and red blood cells obtained from a chicken and carefully washed, was introduced intraperitoneally. The animals were killed at intervals of 2½, 5, 10, 15 and 20 minutes and their anterior mediastinal glands and diaphragms were fixed and studied histologically. In every one some of the ink and red blood cells had reached the glands, the amount increasing with the duration of the exposure. In general the glands after 10 to 15 minutes' exposure in the new-born kitten contained as much ink as that of a fetus at term exposed for 1 to 1½ hours.

It is then obvious that the absorption of particulate matter from the peritoneal cavity via the diaphragmatic lymphatics becomes active at a period at which the fetus begins to make respiratory movements. That the two are closely associated is unquestionable; but the entire dependence of the absorptive function on the respiratory movements has not been demonstrated. With increase in age, and consequent increase in the activity of the movements of the fetus, the amount and rapidity of absorption increases; and after birth there is a still further and even more decided increase despite the fact that many of the fetuses examined were probably within a very few days of parturition. The conclusion then seems warranted that in the fetus the pseudo-respiratory movements are a most important if not the principal factor in starting and maintaining the absorption of particulate material via the diaphragmatic lymphatics. The evidence obtained also indicates that the principal part of the granular material passes through the cytoplasm of the serosal lining cells and not between them, and that very little if any is absorbed by the agency of leucocytes, at least during the first few hours of exposure. Finally, the even distribution of ink granules over the surfaces of the serosal cells and the location of most of the intracellular granules in the region of cytoplasm adjacent to the nucleus, suggest that the particles adhere to the surface quite generally and diffusely, and that the movement of the diaphragm against the liver and other viscera generally tends to force the granules by pressure into the cytoplasm of the cells.

No evidence was obtained regarding the mechanism by which the granules which had been forced into the surface-cells escaped from them,

but it is conceivable that variations in surface tension and other intracellular factors, aided by the respiratory movements would force them out into the spaces between the surface-cells and the lymphatic endothelium.[1] Here it is only necessary to call attention to the probability that the movements of the diaphragm are of considerable importance both in forcing the granules into and through the endothelium, and in assisting the movement of the materials along the lymphatics. The greater delay in the passage of substances from the peritoneal cavity to the mediastinal nodes in the fetuses of earlier stages may, therefore, be assumed to be due to the lack of more vigorous diaphragmatic movements, and it is entirely possible that these movements may be equally as important in the passage of materials through the lymphatic endothelium as through the serosal lining cells.

Elsewhere (5) I have described the histological appearance of the passage of particulate matter, including red blood cells into the lymph vessels, and it has become increasingly evident that in the adult the intracellular route was far more important than the intercellular route advocated by MacCallum, Bolton et al. In general the explanation which seems most plausible from the facts now at hand, is that particles, cells, etc., become adherent to the surface of the peritoneal lining cells without regard to the location of the nucleus or intercellular junctions. That the movements of the diaphragm against the adjacent viscera force the granules into the cell-cytoplasm, but those granules over the nucleus do not enter it but turn aside and pass through the perinuclear zone described above. That this zone may be a very specialized one is indicated by the reports of Clark and Clark (3), Wislocki (11), Cunningham (4) and others on the reactions of vessel endothelium and the lining cells of the serous cavities to vital dyes. The granules and particles which were adherent to the peritoneal lining cells nearer the periphery might be forced into the cytoplasm or between the cells as the case might be. The evidence obtained from the fetuses studied in this manner tends to indicate a preponderance of the intracellular route over the intercellular.

[1] See MacCallum (7), (8) for a description of the anatomical relationship of the lining cells of the peritoneal cavity to the lymphatic endothelium.

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THE INFLUENCE OF TEMPERATURE AND OTHER FACTORS UPON THE TWO-SUMMITED CONTRACTION CURVE OF THE GASTROCNEMIUS MUSCLE OF THE FROG

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Received for publication July 17, 1922

In 1874 Funke (1) discovered that when the gastrocnemius muscle of the frog was in about the middle stage of fatigue, it would produce a contraction curve with two summits upon the application of a single stimulus. The second summit was not as high as the first. Because of the resemblance of the entire curve to the outline of a nose, Funke was led to speak of such a curve as a "Nase." It has, at times, since been spoken of as the "Funkesche Nase."

Fick (2) found that a two-summited curve could be produced by a supra-maximal stimulus applied to the gastrocnemius of the frog. Rollet (3) obtained a two-summited curve by direct and indirect stimulation of the attached gastrocnemius muscle of the frog. Yeo and Cash (4) using very carefully tested apparatus came to the conclusion that "a double summit (or at least a flat-headed curve) is the accurate representation of the normal action of several skeletal muscles under certain conditions."

Graham Brown (5) obtained a two-summited contraction curve of the gastrocnemius muscle when a single stimulus was applied to the intact sciatic nerve. He found that division of the posterior nerve roots or the sciatic nerve might result in the disappearance of the second summit. After this division it always reappeared after a varying length of time. Division of the sciatic nerve with a very sharp instrument had no effect upon the second summit. Division of the peripheral end of the divided sciatic nerve had no effect upon the second summit. Acid applied to the foot of the frog always caused the second summit to disappear; but it reappeared after a short time. Heating the muscle, cooling or warming the nerve caused the second summit to disappear. Strychnine caused both summits of the curve to be increased in height. He also found that, in order to produce the second summit,

it was necessary to avoid injury to the frog in preparation for experimentation. Graham Brown concluded that the normal simple contraction of the gastrocnemius of the frog was double summited.

S. de Boer (6), using the gastrocnemius muscle of the frog, obtained a two-summited curve by indirect or direct stimulation of the muscle. When the rami communicantes to the lumbar nerves had been divided several days, stimulation of the spinal cord produced a simple twitch while stimulation of the muscle itself resulted in a curve which was two-summited. He concluded that the sympathetic nervous system produced and controlled the second summit of the curve. de Boer connects the second summit with muscle tonus.

The following work was undertaken with the purpose of investigating the conditions under which the two-summited curve appears, and, if it proved to be a normal phenomenon, the method of its normal control by the body. In my investigations I have found it desirable to repeat many of the experiments performed by Graham Brown and de Boer.

METHODS. In all experiments, unless otherwise stated, the gastrocnemius muscle of the leopard frog, Rana pipiens, was used. All the animals were pithed, the skin cut around the ankle joint and pulled up over the muscle. The Achilles tendon was cut from its insertion and the gastrocnemius freed from its connection with the bone. The skin was again pulled down over the muscle and the ankle tied securely to the frog board. The knee was held stationary by a needle which was thrust through the knee joint and into the frog board. A small window was cut in the skin over the upper part of the gastrocnemius and a non-polarizable zinc-zinc sulphate electrode placed against the muscle. The non-polarizable electrode and the copper wire running from the tendon to the muscle lever were connected to the secondary coil. The current was obtained from a storage battery of two volts. The lever was weighted with 10 grams placed just beneath the attachment of the muscle to the lever. When the muscle was at rest it supported no weight but care was taken to keep the copper wire taut.

When the frogs had been prepared in the above manner and certain other conditions (to be discussed later) were observed, a two-summited curve always resulted when the muscle or nerve was stimulated with a single maximal induction shock.

THE EFFECT OF MECHANICAL FACTORS UPON THE TWO-SUMMITED CURVE. In all experiments, as mentioned above, the copper wire

which connected the muscle to the muscle lever was kept at the same tension. This was done because it was found that the second summit of the curve was very sensitive to any change in the tension of the wire.

When the tension was decreased the height of both summits decreased but the second decreased much more in proportion than the first. So it was possible in some instances to eliminate almost completely the second summit. This did not mean that the second summit was purely a mechanical phenomenon but rather that it differed fundamentally in behavior from the first summit. When the normal curve exhibited no indication of a second summit, increase of the tension of the wire would not produce a two-summited curve. When by certain manipulations the second summit was caused to disappear an increase in the tension would not bring about its reappearance. So it is seen that this mechanical factor cannot give birth to the second summit but that it may be responsible for its prominence upon the contraction curve.

A decrease in the strength of stimulus or an increase in the load also lowered the height of the second summit more in proportion than the first. So it was possible to cause a disappearance of the second summit by these procedures. As was true of tension, the second summit could not be produced by these manipulations.

When the blood supply to the gastrocnemius muscle was tied off during the course of the experiment, no change in the form or height of the two-summited curve resulted.

THE EFFECT OF TEMPERATURE UPON THE SECOND SUMMIT. de Boer (6), using the gastrocnemius of the intact frog, observed that an elongated cold curve produced by stimulation of the muscle at 16°C. was replaced by a simple twitch when the muscle was heated to 30°C. He believed that the cold curve owed its form to the increased height and length of the second, and the decreased height of the first summit of the two-summited curve. The temperature of 30°C. destroyed the second summit and increased the height of the first. Graham Brown (5) by warming the gastrocnemius with a small Bunsen burner caused the second summit to disappear.

By regulating the temperature of the room and the temperature of the water in which the frogs were kept, it was possible to have the muscle, at the beginning of the experiments, approximately at any desired temperature. During the course of the experiments the temperature of the frogs decreased slowly due to evaporation from

the skin. Thus it was possible to determine the effect of small decreases of temperature upon the form of the curve. The temperature of the muscle was determined, approximately, by a thermometer placed beneath the skin of the back. Since the bodies of the frogs were held in my hand while pithing this temperature was from 1° to 2°C. above

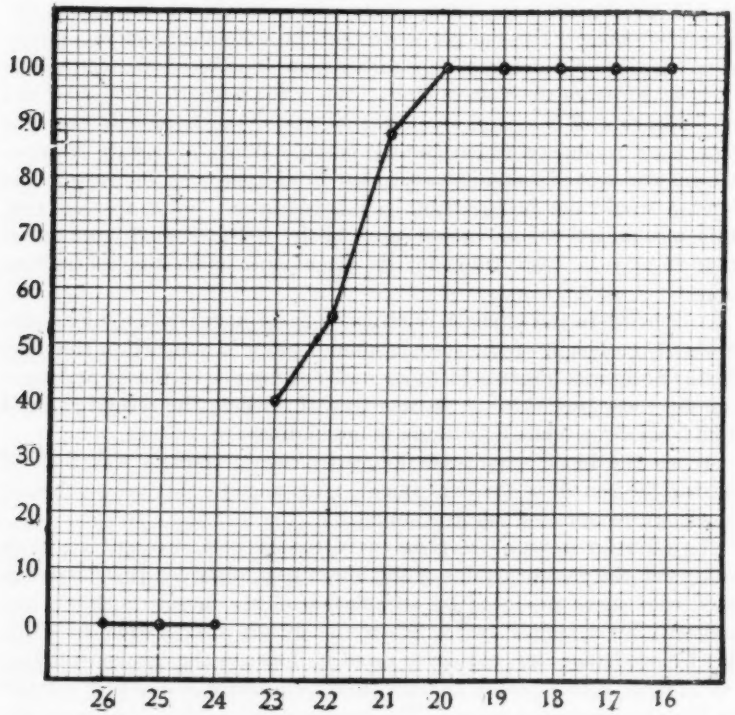

Fig. 1. Curve showing the effect of temperature upon the form of the muscle curve in the intact frog. Ordinates, percentage of experiments in which the muscle curve was two-summited; abscissae, degrees centigrade.

the actual temperature of the gastrocnemius. But as it was not necessary for the purpose of the experiments to record the absolute muscle temperature, this small error was considered unimportant.

Figure 1, which was compiled from about seventy experiments performed during both winter and summer months, illustrates very well

the temperature conditions under which the two-summited curve appeared. It will be noticed that in no experiments did the second summit appear when the temperature of the frog was above 23°C. As the temperature decreased the percentage of two-summited curves increased until between 20°C. and 16°C. all the curves were two-summited.

The height and length of the second summit with reference to the first varied considerably in the different curves. In general, however, the length and height of the second summit was greater the lower the temperature of the muscle. When the temperature of the frog reached about 16°C. the second summit was increased in height and length, so that two summits were not clearly evident, but the whole curve was elongated.

THE EFFECT OF AN INCREASE OR DECREASE IN MUSCLE TEMPERATURE. Figure 2 illustrates the effect of an increase in muscle temperature upon the form of the curve. Here the second summit was well developed but when the muscle was bathed in Ringer's solution at 35°C. for ½ minute the second summit disappeared completely. Three minutes after this the second summit had partially returned and 5 minutes later it had almost reached its original height and prominence. The same effect upon the two-summited curve was obtained when a thermometer registering 35°C. was placed between the skin and the muscle for 1 minute.

The gradual reappearance of the second summit, as shown by figure 2, was caused by the return of the muscle to its original temperature. This was demonstrated by cooling with Ringer's solution or a thermometer at 12°C., a muscle, stimulation of which resulted in a simple twitch. This procedure always produced a two-summited curve which gradually disappeared as the muscle returned to its original temperature (fig. 3).

Effect upon the second summit of warming or cooling other portions of the frog. Since the temperature seemed to be the most important factor, if the two-summited curve was to be produced, it was important to know whether warming or cooling other portions of the frog, than the muscle, had the same effect upon the curve. The opposite leg, the foot of the same side and the body of the frog were bathed in Ringer's solution at 35°C. The same portions were cooled with Ringer's solution at 12°C. The form of the muscle curve was not affected unless the heating or cooling of these parts was so prolonged that the temperature of the gastrocnemius muscle was appreciably affected.

Warming or cooling the muscle tendon, or very small portions of the muscle, with a needle, had no effect upon the two-summited curve.

It is conceivable that a change in muscle temperature could cause a two-summited curve by cooling only the superficial muscle fibers and thus producing a difference in the rate of contraction with reference to those more centrally located. The slower contracting superficial fibers, then, would cause the second summit and the faster contracting central fibers, the first summit. It is apparent, however, that it should be immaterial which of the two groups of fibers is the cooler. Thus the two-summited curve should result both in warming and in cooling the superficial fibers. As has been shown previously, cooling and warming

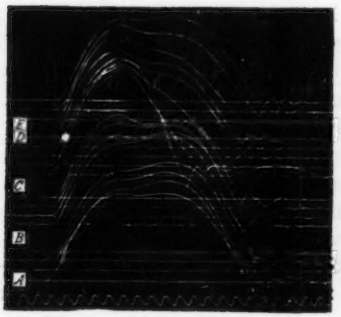

Fig. 2

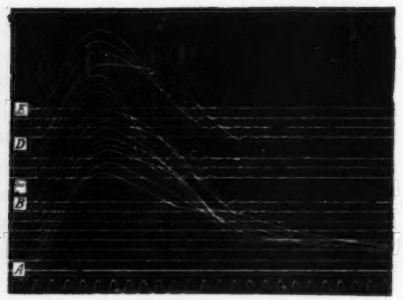

Fig. 3

Fig. 2. The disappearance of the second summit caused by a quick increase in the muscle temperature. *A*, 22°C.; *B*, contralateral leg bathed in Ringer's (35°C.) for 1 minute; *C*, muscle bathed in Ringer's (35°C.) for ½ minute; *D*, return of second summit after waiting 15 minutes; *E*, mechanical stimulation of the foot (temporary decrease in the height of the second summit). *A–B*, 12 minutes; *B–C*, 9 minutes; *C–D*, 15 minutes.

Fig. 3. *A–B*, series of contractions showing the development of the second summit; C, disappearence of the second summit upon division of the sciatic nerve (temperature of frog, 20½°C.); *D*, reappearance of the second summit, after division, upon cooling the muscle with Ringer's solution at 12°C.; *E*, return of contraction to condition in *B* upon return to higher temperature. *A–B*, 20 minutes; *C–D*, 10 minutes; *D–E*, 4 minutes.

with Ringer's solution have exactly the opposite effect upon the form of the muscle curve. Also when the temperature of the frog is sufficiently low the two-summited curve is recorded when the muscle is first stimulated before the temperature has decreased enough to cause a

difference in the rate of contraction of the differently located muscle fibers.

It is, therefore, evident that the change in the form of the muscle curve is produced by the altered actual temperature of the muscle at the time of stimulation. If the temperature is below about 16°C., a typical cold curve is always produced. If it is above approximately 23°C., the simple curve is just as constantly produced. The two-summited curve writes the normal simple contraction of the gastrocnemius muscle between the temperatures of 16° and 23°C.

REMOVAL OF THE EFFECT OF THE SPINAL CORD. *Division of the sciatic nerve.* The sciatic nerve was not disturbed until just before division, when it was exposed by carefully dividing the muscle which lay over it. The nerve was always divided with sharp scissors and the temperature of the frog, at the time of division, was carefully noted.

In many experiments division of the sciatic nerve had no effect upon the second summit of the muscle curve while in others division with the same instrument and in the same manner caused partial or complete disappearance of the second summit (fig. 3). It soon became evident that this difference in effect was closely associated with the temperature of the frog. Division of the sciatic nerve caused a disappearance of the second summit only when the temperature of the muscle was 18°C. or above and division of the nerves when the temperature of the frog was below about 18°C. had no effect upon the second summit. The range of temperatures affecting the results of such division in a series of experiments is as follows:

Disappeared	22	21	21	20½	20½	20	19¾	19½	19	19	18¾	18	18
Reduced										19	19	18½	18
Not affected	18	18	17¾	17¾	17	17	16	16	16	15	15		

In four experiments the second summit was only decreased in height and length when the temperature of the muscle was around 18°C. Evidently there is no sharp boundary line on one side of which division of the sciatic nerve causes a complete disappearance of the second summit and on the other side no effect upon the second summit. The two-summited curve which can be affected by division of the sciatic nerve usually cannot be distinguished from the other by its form and shape.

Paralysis of the spinal cord by a 4 per cent solution of alypin which was injected into the spinal canal caused the disappearance of the second summit when the muscle temperature was above about 18°C.

Division of the posterior nerve roots. It was rather difficult to determine the effect of division of the posterior nerve roots upon the two-summited curve because exposure of the spinal cord, preparatory to division, in most instances, caused the second summit of the curve to disappear and, of course, prevented the performance of the experiments. This may have been caused by spinal shock. The posterior roots of the 7th, 8th and 9th nerves of the homolateral side were exposed by removal of the portion of the vertebral column over them and the wound was closed with stitches. After 24 hours the two-summited curve resulted when the muscle was stimulated. Contractions were recorded after reëxposure of the spinal cord and after the posterior roots were lifted with a glass hook, and these showed no change in the form of the curve; but division of the posterior roots caused a disappearance of the second summit. However, as upon division of the sciatic nerve or paralysis of the spinal cord, division of the posterior nerve roots was ineffective unless the temperature of the frog was above about 18°C.

REAPPEARANCE OF THE SECOND SUMMIT AFTER DIVISION OF THE SCIATIC NERVE. Since division of the posterior nerve roots, sciatic nerve, and paralysis of the spinal cord causes the second summit of the curve to disappear, it would seem that the second summit is caused by a tonic action of the spinal cord. However, as was observed by Graham Brown (5), in most instances after division of the sciatic nerve, the second summit returns after a varying length of time, which would seem to show that the division had merely a temporary inhibitory action upon the second summit of the curve.

By noting the temperature of the muscle after division of the sciatic nerve it was discovered that as long as the temperature of the frog remained the same or did not drop below about 18°C., the second summit did not reappear even after several hours. When the temperature of the frog increased after division, as was true of frogs which had been kept in an ice chest, the second summit did not reappear. When, however, the frog's temperature dropped below about 18°C., the second summit always reappeared and the time elapsing before its return depended upon the rapidity of the decrease in muscle temperature. The second summit always reappeared immediately when the muscle was bathed in normal salt solution at 12°C. (fig. 3). The second summit reappeared, after paralysis of the spinal cord and division of the posterior nerve roots, under the same conditions as after division of the sciatic nerve.

Graham Brown stated that division of the sciatic nerve, with very sharp scissors, had no effect upon the second summit, presumably because of absence of mechanical stimulation. He did not record the temperature of the muscle at the time of division but one would judge from the form of the curves, used to illustrate the experiment, that the temperature of the muscle must have been below 18°C. In all of my experiments, the division of the nerve was accomplished with sharp scissors and a minimum stimulation of the nerve but disappearance of the second summit always resulted when the temperature of the frog was proper.

The above results furnish good evidence that at a temperature below about 18°C. the second summit is muscular in origin; above 18°C. the second summit is produced reflexly.

THE PATH OF THE IMPULSES WHICH CAUSE THE SECOND SUMMIT. Since the second summit is produced reflexly it is important to know the course of the impulses concerned, namely, their path in the spinal cord, the origin of the afferent impulses and their path to the spinal cord, and the course of the efferent impulses to the muscle.

In the spinal cord: The segment in the spinal cord through which these impulses passed was determined by successive divisions of the spinal cord from the anterior portion, posteriorly. Five experiments of this kind were performed. The spinal cord was not exposed before division but the intact vertebral column was divided with sharp scissors. If the divisions are made exactly at right angles to the cord, and if the cuts are clean, no marked stimulation of the cord occurs. The divisions were made between the emergence of the 4th and 5th, 5th and 6th, and 6th and 7th spinal nerves. Division of the spinal cord in the first two locations had no effect upon the second summit of the curve. Division between the emergence of the 6th and 7th nerves, and in one experiment, division at the level of the 6th nerve, caused complete disappearance of the second summit. In all of the experiments an examination of the cord showed that the divisions which caused the disappearance of the second summit occurred just at the emergence of the roots of the 8th nerve. This located the impulses concerned in the production of the second summit, in the last three segments of the spinal cord, the 8th, 9th and 10th.

In the spinal nerves: Division of the 7th, 8th, 9th and 10th spinal nerves of the contralateral side had no effect upon the height or form of the second summit.

The 7th, 8th, 9th and 10th nerves of the homolateral side were divided individually in fifteen experiments. Division of the 7th and 10th nerves had a negative effect in all of the experiments.

The usual effect of individual division of the 8th and 9th nerves is shown by figure 4. In all of the experiments division of the 8th nerve either had no effect upon the second summit or caused a slight temporary reduction in the height. Division of the 9th nerve always caused a complete disappearance of the second summit when the temperature of the frog was above 18°C.

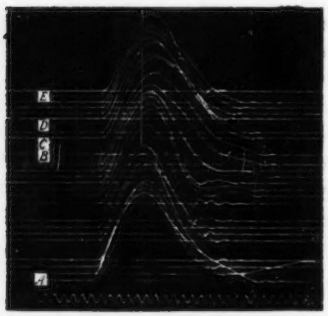

Fig. 4. The effect upon the second summit of division of the rami communicantes. *A–B*, development of the second summit (temperature of frog, 22½-19¼°C.); *C*, division of rami to the 7th, 8th and 9th nerves; D, division of the 8th nerve; *E*, division of the 9th nerve (temperature of frog, 18½°C.). *A–B*, 50 minutes, *B–D*, 5 minutes; *D–E*, 12 minutes.

In the nerve roots: Individual division of the posterior nerve roots of the 7th, 8th and 9th nerves had the same effect as division of the individual spinal nerves. So the afferent impulses concerned in the production of the second summit must be carried principally in the posterior root of the 9th nerve.

The location of the path of the efferent impulses in a definite anterior nerve root was not accomplished by direct experimentation because it was very difficult, if not impossible, to expose these roots without causing the second summit to disappear. However, unless the efferent path of the impulses is over antidromic fibers in the posterior root of the 9th nerve, which is improbable, it must be principally over the anterior root of the 9th nerve.

The location of the receptors: It has been shown that temperature can produce the second summit only by acting upon the gastrocnemius muscle. This in itself furnishes good evidence that the receptors are located in the muscle itself. In order to test this the following experiment was performed: The skin of the leg was painted with a 2 per cent cocaine solution. After about 5 minutes the skin was removed from the leg. A curve was recorded which showed that this procedure had had no effect upon the second summit. The leg was then amputated at the knee joint and branches of the sciatic nerve supplying the thigh

muscles were divided. This also had no effect upon the curve. The 7th, 8th, and 10th nerves were divided with no effect. Subsequent division of the 9th nerve caused complete disappearance of the second summit.

That the receptors are not located in the tendon may be shown by placing the hook connecting the muscle with the lever above the tendon. Division of the tendon then has no effect upon the second summit.

The part played by sympathetic nerves in the production of the second summit. The above experiments located the afferent and efferent path of the impulses principally in the 9th nerve. Since the rami of the 8th and 9th spinal nerves can be divided easily it is possible to determine whether the impulses causing the second summit are carried over sympathetic fibers.

The frog was prepared with intact circulation and placed upon its back on the frog board. After the presence of the second summit had been demonstrated by several stimulations of the muscle, the abdomen was opened by a mesial slit, the viscera shoved to the opposite side exposing the rami communicantes to the 7th, 8th and 9th nerves. This operative procedure had no effect upon the second summit of the curve as was shown by taking a record of another muscle contraction. Then the rami of the 7th, 8th and 9th nerves were divided. The effect of the division was determined by recording a contraction every few minutes (fig. 4). Ten experiments were performed. In none did division of the rami to these nerves have any effect, while subsequent division of the 9th nerve or the sciatic caused a complete disappearance of the second summit.

In another series of experiments the rami communicantes were divided, the spinal cord in the lumbar region was exposed and records taken of the form of contraction of the gastrocnemius muscle resulting from stimulation of the spinal cord, and from stimulation of the muscle directly. The form of the curves in each case was the same in all of the experiments. When the stimulation of the muscle caused a two-summited curve, so did stimulation of the spinal cord. When the temperature of the frog was above about 23°C. stimulation of both the cord and muscle produced a simple twitch.

No evidence, therefore, was obtained which would substantiate de Boer's results that division of the rami communicantes causes the second summit to disappear or that the second summit is caused by a single stimulus applied to sympathetic nerve fibers.

The course of the impulses, then, which reflexly cause the second summit of the muscle curve, is as follows: The endings of the afferent fibers are located in the gastrocnemius muscle and the afferent impulses reach the spinal cord principally over the posterior root of the 9th spinal nerve; their path in the spinal cord is in the last three segments. The efferent impulses probably leave the spinal cord principally over the anterior root of the 9th spinal nerve and take the same path to the muscle as the spinomotor fibers.

THE EFFECT OF REPEATED STIMULATION UPON THE FORM OF THE MUSCLE CURVE. Repeated stimulation of the nerve fibers concerned in the reflex production of the second summit always caused the appearance of the second summit, if it was absent, or an increase in height and length. The amount of change in the form of the curve varied directly with the length of stimulation. After removal of the stimulating current the curve regained the original form after several minutes.

The second summit, however, which resulted from repeated stimulation was not produced by the same mechanism which caused the normal summit, because division of the sciatic nerve did not cause its disappearance in any instance.

The second summit was not affected by repeated stimulation unless marked contraction of the gastrocnemius muscle occurred. Therefore, the curve was probably analogous to the "Nase" seen by Funke (1) in the middle stage of fatigue. Muscle curves obtained by Lee (7) after partial fatigue of frog's muscle and after injection of lactic acid have the same form as my curves. So it seems most probable that the second summit was caused by the action of products of contraction.

This ability of substances in the acting muscle to produce the second summit made it impossible to determine whether the nerve fibers which were concerned in the reflex production of the second summit were effectively stimulated, but it is interesting that the expected result, an increase in the height of the second summit, was gotten when the nerves to or from the muscle were stimulated, even though the second summit was apparently muscular in origin.

THE INFLUENCE OF TEMPERATURE UPON THE SECOND SUMMIT PRODUCED BY REPEATED STIMULATION. When a two-summited curve or an elongated fatigue curve was produced by repeated stimulation of the muscle, warming the muscle to a temperature of 26° to 30°C. caused the form of the curve to be changed to a simple twitch (fig. 5). The amount of increase in temperature necessary to accomplish this

varied directly with the length and rate of stimulation. This result was obtained equally well after division of the sciatic nerve and after the gastrocnemius muscle had been paralyzed by the action of curare.

When the simple twitch appeared upon heating the muscle the curve always returned to its original form after several minutes. This was caused by the return of the muscle temperature to its original level, since when the muscle was cooled to about 18° to 19°C. by a cool thermometer, the curve always returned to its original form within 30 seconds.

An elongated curve, similar to the ones produced by repeated stimulation of the nerve or muscle, resulted when a small amount of lactic acid was injected into the aorta. When the temperature of the muscle was raised to 30°C. this curve was replaced by a simple twitch. When the muscle returned again to its original temperature the elongated curve was recorded.

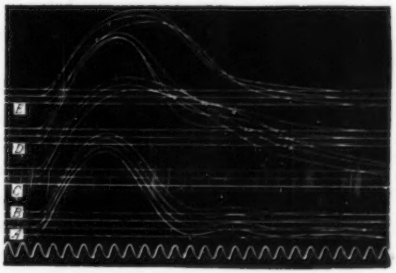

Fig. 5. *A–B*, temperature of frog, 20°C.; *C*, production of two summited curve by repeated stimulation of muscle; *D*, destruction of the second summit upon heating the muscle to 26°C.; *E*, return of the elongated curve upon cooling the muscle to 20°C. *A–B*, 10 minutes; *C–D*, 4 minutes; *D–E*, 3 minutes.

When a muscle which writes a two-summited curve is repeatedly stimulated the change that first occurs is an increased prominence of the second summit. As the muscle is further stimulated it can be seen that the second summit, increasing in length and height at the expense of the first, is the principal factor in the production of the elongated fatigue curve. When a curve of this type is produced a decrease in the tension on the wire to the muscle lever will change the flat-topped fatigue curve to one which shows clearly two summits. Therefore, it would seem that the factor which is responsible for the elongated fatigue curve is also the cause of the second summit produced by repeated stimulation. All available evidence points to the products of muscular contraction, and in particular the acid products as the cause of the flat-topped fatigue curve.

Meyerhof (8) found that when a muscle was fatigued at 5°C. and then brought to a temperature of 20°C. a series of contractions could be again elicited. However, if a muscle fatigued at 20°C. was cooled to 5°C. no contractions resulted from stimulation. He also determined the amount of lactic acid in muscles fatigued at high and low temperatures in the absence of oxygen. In all instances there was much more lactic acid present in those muscles which had been fatigued at high temperature. Therefore, it would seem that at a high temperature lactic acid is not as active in the production of fatigue as at a lower temperature. In my experiments, then, it is possible that the change in the form of the curve with increased temperature was dependent upon this decreased ability of the lactic acid to act upon the muscle substance.

THE ACTION OF STRYCHNINE. Graham Brown found that after the injection of small amounts of strychnine into the dorsal lymph sac, both the first and second summits were increased in height.

In my experiments, $\frac{1}{40}$ to $\frac{1}{50}$ mgm. of strychnine nitrate was injected into the spinal canal or the dorsal lymph sac after normal muscle curves had been recorded. By comparing the curves, which were recorded after the injection, with the normal ones the effect of strychnine was determined. The following results were obtained:

1. When the curve was two-summited, strychnine caused a marked increase in the height of both the first and second summits. The amount of this increase was in the same proportion for each summit (fig. 6).

2. When the temperature of the muscle was below 23°C. and a simple twitch resulted when the muscle was stimulated, strychnine in some experiments caused the second summit to appear, and as in 1, both summits were much higher than normally.

3. In several experiments the posterior nerve roots, of the 8th and 9th spinal nerves, were exposed before the injection of strychnine. After strychnine had acted, division of these nerves caused the complete disappearance of the second summit and the return of the first summit to its original height. Strychnine had no effect upon the muscle curve when the posterior nerve roots had been divided before its injection.

4. The second summit produced by the action of strychnine disappeared completely when the muscle temperature was raised by bathing it in Ringer's solution at 35°C. The height of the first summit was increased.

As shown by the above results, strychnine produces or increases the height of the second summit, provided the temperature of the muscle is below a certain maximum. Since it has no effect upon the muscle curve after division of the posterior roots of the 8th and 9th nerves, strychnine must augment the effectiveness of impulses which reach the spinal cord from the muscle, or change the nature of the afferent impulses. Consequently, since temperature above about 23°C. seems to block the afferent or efferent impulses, which cause the second summit, at some place in the muscle, it would be expected that it would have the same effect upon the second summit caused by the injection of strychnine.

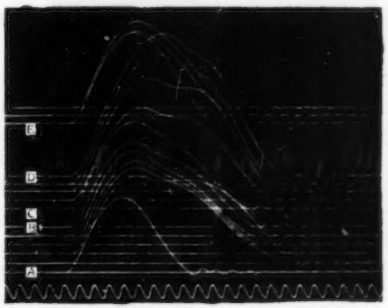

Fig. 6. The effect of strychnine. *A–B*, temperature of frog, 22–20¾°C.; *C*, injection of $\frac{1}{10}$th mgm. of strychnine nitrate into the dorsal lymph sac; *C–D*, increase in height of both the first and second summits; *E*, division of the sciatic nerve. *A–B*, 15 minutes; *C–D*, 11 minutes.

Many attempts were made to produce the second summit by other methods, besides temperature, strychnine and repeated stimulation of the muscle, but all were ineffective.

REFLEX INHIBITION OF THE SECOND SUMMIT. If the tonic action of centers in the spinal cord is the causative factor in the production of the second summit, it should be possible to depress the action of these centers by stimulation of certain sensory nerve fibers and thus cause a temporary disappearance of the second summit. It was discovered that when the temperature of the frog was above about 18°C. the second summit of the curve could be inhibited in the following ways:

Mechanical stimulation of the sciatic nerve. When the sciatic or the 9th nerve was stimulated by pulling upon a ligature or by gently rubbing with a steel hook, the second summit decreased in height or disappeared completely, depending upon the intensity of the stimulation (fig. 7).

The second summit always returned, when the mechanical stimulation was weak, in 2 to 5 minutes. This return was not necessarily associated with a decrease in muscle temperature. Consequently division of the nerve or mechanical stimulation were, in most cases,

still effective. After very strong mechanical stimulation of the sciatic, in many instances, the second summit did not return until the temperature of the frog dropped below 18°C.

When the temperature of the frog was close to 23°C. this reaction to mechanical stimulation was so delicate that many times the handling of the sciatic nerve necessary for its exposure resulted in a temporary disappearance of the second summit.

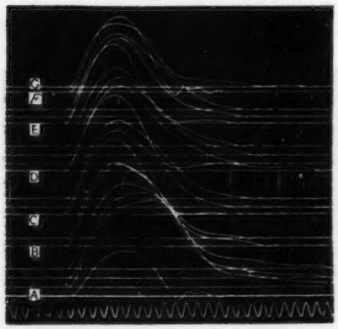

Fig. 7. The effect upon the second summit of electrical stimulation of the central end of the 8th nerve and mechanical stimulation of the 9th nerve. *A*, temperature of frog, 19°C.; *B*, division of the 7th and 8th nerves; *C*, *D*, stimulation of the central end of the 8th nerve resulting in a temporary disappearance of the second summit; *E*, mechanical stimulation of the intact 9th nerve resulting in a temporary disappearance of the second summit; *F*, division of the 9th nerve. *A–B*, 7 minutes; *B–C*, 6 minutes; *C–D*, 5½ minutes; *D–E*, 5½ minutes; *E–F*, 4 minutes; *F–G*, 60 minutes.

It might be thought from the above that the disappearance of the second summit, after division of the sciatic nerve, is caused by the mechanical stimulation of the nerve with the scissors. However, by taking the contraction of the gastrocnemius muscle as an index of the intensity of the stimulation of the nerve, it was found that division of the nerve in most experiments was accomplished with less stimulation than that necessary to cause a disappearance of the second summit. Also, in many experiments, division of the sciatic nerve resulted in a disappearance of the second summit after mechanical stimulation had been ineffective.

Mechanical stimulation of the sciatic nerve could produce its effect either by stimulation of depresser nerve fibers to the spinal cord, or by efferent fibers which had an inhibitory effect upon the second summit. The latter possibility could be tested by mechanical stimulation of the intact anterior roots of the 8th and 9th nerves, but because of the difficulty in exposing these roots without causing the disappearance of the second summit this was not accomplished.

Stimulation of the central end of the 8th spinal nerve. If mechanical stimulation of the sciatic nerve produced its effect by acting upon af-

ferent nerve fibers, then stimulation of the posterior roots of the 8th or 9th nerves would cause the second summit to disappear.

As has been mentioned before, exposure of the lower portion of the spinal cord causes a prolonged disappearance of the second summit. This, of course, made it impossible to test satisfactorily the effect of stimulation of the posterior nerve roots upon the second summit. Division of the 8th spinal nerve had no effect upon the second summit. Therefore, it was possible by stimulating the central end of this nerve to determine the effect upon the second summit of impulses over the posterior nerve root.

Ten experiments were performed. After division of the 7th and 8th spinal nerves the central end of the 8th was stimulated for 10 to 20 seconds with a tetanizing current which was just perceptible to the tongue. A current of this strength usually caused no contraction of the gastrocnemius muscle, but in some instances a few weak isolated twitches of the muscle occurred.

In all of the experiments the second summit either disappeared temporarily or was decreased in height. Division of the 9th nerve, after stimulation of the central end of the 8th, always caused a more complete and permanent disappearance of the second summit (fig. 7).

Chemical and electrical stimulation of the foot. Stimulation of the foot with 2 per cent sulphuric acid caused the second summit to disappear when the temperature of the frog was above about 18°C. The second summit returned in a few minutes providing the stimulation was not too strong or too prolonged. After this return division of the sciatic nerve caused a more permanent disappearance of the second summit.

Stimulation of the foot, for several seconds, with a strong tetanizing current had the same effect upon the second summit as stimulation of the foot with acid. Acid, on the foot of the contralateral side, had but very little if any effect upon the second summit of the curve.

Usually the second summit did not disappear unless the stimulation of the skin was very strong. In some frogs, however, light stimulation was effective. In one experiment the second summit was decreased in height when the skin of the shank or foot was rubbed lightly with a small wad of cotton. Stimulation of the thigh or back, however, had no effect upon the curve.

THE TWO-SUMMITED CONTRACTION CURVE OF THE TRICEPS MUSCLE. The triceps muscle of the frog was used in several experiments. It was found that this muscle responded to a single stimulus with a two-summited contraction under certain conditions. These were similar to

those necessary for a two-summited curve of the gastrocnemius except that the temperature of the muscle was usually slightly lower. Under the proper temperature conditions division of the sciatic nerve caused a disappearance of the second summit.

THEORETICAL DISCUSSION. *a. Discussion of the mechanism of the normal stimulation of the afferent nerve endings.* All evidence presented above seems to indicate that the second summit, when it apears between about 18°C. and 23°C., is caused by a tonic action of the spinal cord which is produced by continual afferent impulses from the muscle itself.

Since the reflex second summit occurs only at certain muscle temperatures, it is evident that temperature is a necessary condition for the stimulation of the receptors in the muscle. Therefore, the following question suggests itself. Is temperature the effective stimulus for the receptors or is a certain muscle temperature merely necessary for the action of some substance which in turn is the effective stimulus for the afferent nerve endings?

As was discussed on pages 272 and 273, the two-summited curve produced by repeated stimulation is probably caused by the acid products of contraction. Some evidence was also presented that the disappearance of the second summit, upon warming the muscle, was caused by the decreased activity of these products of contraction. If the latter statement is true, then an increase in the second summit could be produced either by increasing the actual amount of contraction products or by lowering the temperature of the muscle. In either case the form of the curves should be the same. This is, indeed, true except that the relaxation phase of the curve produced at low temperature is not so prolonged as the relaxation phase of the typical fatigue curve. Schenck (9) showed, however, that the relaxation of a muscle occurred more slowly in proportion with the scarcity of reserve materials in the muscle; and when a muscle was fatigued by the injection of lactic acid a curve resulted, upon stimulation, which had the same form as a curve produced by stimulation of a fresh muscle at a low temperature.

Another point of interest in this connection is the action of veratria on the muscle curve at different temperatures. Brunton and Cash (10) found that when the temperature of the frog's gastrocnemius was around 15°C., small doses of veratria had no effect upon the form of the muscle curve, but as the temperature was increased the elongated relaxation which is typical of the simple contraction of a veratria-

poisoned muscle became more pronounced and reached its maximum at about 25°C. This is just opposite to the effect of temperature upon the two-summited curve. Since products of contraction antagonize the action of veratria, and if their activity is increased as the temperature decreases, the above action of temperature on the veratria curve is explainable.

From the above evidence it would seem very probable, then, that the ability of these contraction products to produce the second summit depends upon the temperature of the muscle. Of course, the curves discussed above are entirely muscular in origin. Now the two-summited curve which appears between 18° and 23°C. is produced reflexly. However, the two curves are very similar in appearance and in their reaction to temperature. Moreover, when the muscle is cooled the double-summited curve which is produced reflexly becomes purely muscular. Therefore, it seems reasonable to suppose that in both curves the causative factor of the second summit is the same but that in the reflex curve the acid products stimulate the afferent nerve endings in the muscle while in the other they act upon efferent nerve endings or a contractile substance in the muscle itself. Whether the products would act at one or the other of these places would depend upon the concentration and also upon the activity of these products. Above 23°C. their activity would be so decreased as to have no effect. Between 18° and 23°C. they would act upon afferent nerve endings in the muscle; below 18°C. they would act upon the muscle itself.

b. The cause of the second summit and its relation to muscle tonus. When the muscle is warmed the second summit decreases in height while the first summit increases in height. When the muscle is cooled the first summit decreases in height while the second increases. It seems to me that this opposite effect of temperature upon the two summits in all probability means that the second summit is not caused by the contraction of the muscle fibrils but by some other contractile substance in the muscle.

Since reflex tonus and the second summit obtained between 18° and 23°C. are caused by a tonic discharge from the nerve centers in the spinal cord, it is possible that they are caused by the same contractile substance in the muscle or at least are very closely associated phenomena. Most manipulations which caused a disappearance of the second summit are known to cause a decrease of muscle tonus, for example, division of the nerve to the muscle, division of the posterior nerve roots, heat and stimulation of the foot with acid. An increase

in the height of the second summit occurred with cold, strychnine and repeated stimulation of the muscle or nerve. If these affect muscle tonus at all they would increase it. Therefore, I am inclined to believe that at least between 18° and 23°C. the presence of the second summit upon the curve is an indication that the muscle is in tone.

SUMMARY

The normal simple contraction curve of the attached gastrocnemius muscle of the pithed frog is double-summited, when the frog's temperature is between about 16° to 23°C. When the temperature of the frog is above about 23°C. the curve is single-summited; below about 16°C. the two summits are fused to form an elongated, flat-topped, cold curve.

The second summit of the two-summited curve is muscular in origin when the temperature of the frog is below about 18°C. It may be increased in height by repeated stimulation of the muscle or nerve and decreased in height by an increase in muscle temperature.

When the temperature of the frog is between about 18°C. and 23°C. the second summit may be increased in height by: small doses of strychnine; repeated stimulation of the muscle or nerve; a slight decrease in muscle temperature. It may be caused to disappear by: division of the sciatic or the 9th spinal nerve; division of the posterior root of the 9th nerve; mechanical stimulation of the sciatic nerve; chemical or electrical stimulation of the foot of the homolateral side; electrical stimulation of the central end of the 8th spinal nerve; an increase in muscle temperature.

When the temperature of the frog is between 18° and 23°C. the second summit is caused by a tonic discharge of nerve centers located in the last three segments of the spinal cord. The tonicity of these centers is dependent upon the constant inflow of afferent impulses which arise in the gastrocnemius muscle. The impulses concerned in the production of the second summit do not travel over sympathetic nerve fibers.

The possibility is discussed that between 18° and 23°C. the products of muscular contraction are the effective stimuli for the afferent nerve endings in the muscle.

Available evidence indicates that the second summit is caused by a contractile substance in the muscle which is different than that which causes the first summit.

The second summit of the curve seems to be very closely associated with muscle tonus.

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THE ACTIVE RESPONSE OF CAPILLARIES OF FROGS, TADPOLES, FISH, BATS AND MEN TO VARIOUS FORMS OF EXCITATION

I. Excitation by Electricity

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Received for publication July 17, 1922

The purpose of the experiments reported in this paper was to ascertain whether capillaries actively respond to localized stimulation, and it has been found that this is the case.

Using Lombard's method, one can see the capillaries of the human skin. Studies of the response of capillaries to excitation are no longer limited to animal tissues, nor will one be satisfied with the color reaction, as it is now possible to obtain a direct observation of the human capillary, and, under certain conditions, as Danzer and Hooker (12) found, even to see the movement of the blood corpuscles in the human body. Nevertheless, it is of value to study the behavior of the capillaries of animals for each tissue has its advantages and peculiarities. Although it has been suggested from time to time that capillaries may play an active part in propelling the blood, the generally accepted view has been that capillaries are entirely passive, and that the pressure and rate of flow of blood through them is controlled by the general arterial blood pressure, and the contraction and dilatation of the arterioles supplying the region. In recent years, however, evidence is accumulating which shows that capillaries can actively change their caliber, that they are controlled by nerves, and that they may play an important part, independently of arterial reactions, in the regulation of the distribution of the blood.

It is well known that there is an accelerated blood flow through an organ during its functional activity. Here what is important is the increased amount of blood passing through the capillaries, for in the capillaries takes place the exchange of gases, hormones, hormosomes and other materials needed for metabolism, and sometimes the distribu-

tion of substances, needed for preventive or curative purposes. For this reason, in a functioning organ, not only the size of individual capillaries, but also the number of capillaries through which the blood is flowing is increased to meet the need. Thus Krogh (1) has found that in resting muscles of frogs many capillaries are occluded, that as a result of activity the number of patent capillaries is enormously increased, and that in working muscles capillaries show a greater diameter. It is probably true of the human body that the nutrition of living cells depends largely on the efficiency of that part of the blood vessels with which the tissue function is most intimately concerned.

Not only does the condition of the capillaries of a tissue help to determine the blood supply to that part but the size of the capillaries has a marked influence on the general circulation. Examining the web of the frog, for example, the observer will note how great is the number of capillaries. He will also appreciate how large a proportion of the total blood can be contained in the capillary region of the vascular system, and how an increase in the diameter of the capillaries, if it occurs in a large part of the body, would absorb a large fraction of the total blood. The amount of the blood driven out by the heart in each beat, depends upon the amount entering its ventricles in diastole, so that if there is any accumulation in the periphery, the circulating blood is decreased and the blood pressure falls. This would be very significant if the enormous capacity of the capillaries were increased by some means, for instance, by toxic products from injured tissues, as mentioned by Bayliss (2), and Lee (3), and in the case of "histamine shock," by Dale and Laidlaw (4). Recently the direct observation of capillaries has stimulated a clinical interest in the condition of capillaries in many diseases. In the past three years many papers have appeared dealing with capillary pressure, and the circulation of blood in capillaries in diseased conditions, such as nephritis, arterio-sclerosis, shock and cardiac decompensation.

Any condition altering the nutrition of a tissue must have some vital relation to the capillaries and probably there are not only close interrelationships between capillary efficiency and certain diseases, but also between the latter and the active response of capillaries. It is hoped, therefore, that studying the active response of capillaries to excitation, may throw some light on the index of capillary efficiency, and consequently on the field of medicine.

Although the work of a number of observers has made it probable that the endothelial cells of the walls of the capillaries play an active

part in the regulation of the circulation, the view is not universally accepted. Very recently, for example, Friedlander and Lenhart (5) pointed out that "overwhelming proof has not yet been assembled in favor of the independent contractility of capillaries." There is no definite evidence that the capillaries of man are capable of actively changing their caliber. Any contribution giving evidence on this important question should be of value, and especially, because of its clinical significance, information is needed concerning the response of human capillaries to stimulations.

Structure of capillaries. Apparently some experimenters in this field have failed to make a sharp distinction between arterioles, capillaries and venules, and it is necessary that the writer should state at the outset his conception of the term capillary. Ranvier's view, that the capillary begins where the muscular coat of the artery ends and ends where the muscular coat of the vein begins, is probably correct, except that the venule should be considered to begin, as Hooker (38) points out, when the endothelial cells of the capillary begin to acquire a connective tissue sheath. Capillaries are therefore to be defined as tubes composed of bare endothelial cells, which are a continuation of lining cells of the arterioles and venules. The form of the endothelial cells varies according to the diameter of the capillary. Usually they are of irregular, oblong shape. The protoplasm of the endothelial cells is homogeneous, or presents some granulations in the neighborhood of a nucleus.

"The capillaries accommodate themselves to the shape of the elements of tissues or organs in which they are situated. In the muscle and nerves, they form a network with oblong meshes" (6). In the papillae of the human skin, capillaries are arranged in the shape of loops.

Schäfer (8) states that capillaries receive nerve fibers, which form a plexus of fibrils in close relation to the endothelial cells of which the walls of the vessels are composed, but contacts between the endothelial cells of blood capillaries and nerve-fiber endings have not been generally recognized, although there is physiological evidence that capillaries are controlled by nerves. Hooker saw contraction of capillaries of the ear of the cat on excitation of the cervical sympathetic nerve. Krogh (25) mentions that there are nerve endings in the capillary walls, and in a recent paper Krogh and others (39) state that capillaries and arteries in skin and muscle of the hind legs in frogs are innervated through sympathetic fibers, and through posterior root fibers.

The work of others. The review of the literature given by Hooker (30), (38), is so complete that only a summary of some of the evidence relating to the active response of capillaries to direct excitation is necessary.

Certain authors have accepted the idea that change in the size of capillaries is merely passive. For example, Natanson (9) concludes that capillaries cannot be filled except under the influence of arterial pressure. Without the latter, the capillaries collapse passively. Employing the method of von Kries (10) Natanson took for his criterion the complete blanching of the skin. The inadequacy of this method had been pointed out by Lombard (11), who found that the pallor of the skin is not necessarily associated with a cessation of flow of blood in the superficial capillaries. Danzer and Hooker (12) also failed to confirm his observation, and believed that the inadequacy of the criterion used by Natanson was sufficient to explain his results.

On the other hand, there is considerable evidence which goes to show that capillaries actively respond to stimulation. In 1865 Stricker (13) observed capillaries in the nictitating membrane of the frog to dilate and to constrict. In the following year, Stricker (14) modified his technique by examining the isolated nictitating membrane in aqueous humor. When the membrane was exposed to ammonia vapor for three or four seconds, and then examined under the microscope, he saw the capillary lumen almost disappear and then enlarge. He also obtained positive results by electrical stimulation. When the capillaries contracted they became invisible.

Golubew (15) confirmed the observation that capillaries change their size under various conditions, and regarded these changes as due to certain spindle elements. Rouget (16) described perivascular cells which he regarded as the agents causing the alteration of the size of the capillary. Severini (17) claimed that the application of CO_2 caused capillaries to dilate, while oxygen made them contract. But Severini's experiment was not confirmed by Tarchanof (18) nor by Roy and Mehring (19).

Golubew's view was adopted by Tarchanof who employed alcohol, ether, ammonia, ferric chloride, acetic acid, and heat to stimulate capillaries. These procedures, according to him, aroused the so-called spindle elements to swell, thus occluding the lumen.

Roy and Brown (20) attempted to measure the blood pressure in capillaries. They were convinced by their observations that capillaries possessed active contractility. They saw that local anemia, produced

by compression of an area, was followed by hyperemia, accompanied by a dilatation of arterioles, venules and capillaries. Dilatation of capillaries was also seen on the application of chloroform. They concluded that it was not the spindle elements of Golubew, but "it is by the contractility of the capillary wall as a whole, that the diameter of the vessel is changed." They also mentioned that the congestion which followed the compression of a portion of the web was an active change, because the dilatation of the capillaries was greater than could be explained by the increase of their internal pressure.

Roy and Brown gave further evidence that the contraction and dilatation of capillaries were not merely caused by changes in pressure. They found that there was little difference in the size of the capillaries before and after the frog's leg was amputated. By the application of chloroform and tapping the abdomen, the capillaries remained dilated with a low intracapillary pressure. Again, the effect on the caliber of the capillaries, of raising the extra-capillary pressure was usually very slight.

Krogh (1) has pointed out that the pressure necessary to force muscle capillaries to open, when they are actively contracted, is very much higher than the normal arterial pressure, and that capillaries do not contract measurably by elasticity when the pressure is reduced by closing or cutting the arteries. This is better understood if one recalls that though large thin walled rubber tubes collapse when internal pressure is withdrawn, this is not true of smaller tubes, and tubes as small as capillaries in spite of the delicacy of the walls, might keep open even with no internal pressure.

A paper by Steinach and Kahn (21), though largely concerned with repetition of earlier work, is interesting. They employed the cut nictitating membrane of the frog. Steinach and Kahn saw the perivascular cells described by Rouget (16), and adopted the view that the perivascular elements were responsible for capillary constriction.

If a blunt pointed instrument is drawn across the skin, a white line is left, which turns to red in a few seconds. Marey explained it as due to a localized contraction and dilatation of capillaries. But Bloch (23) thought there was only evidence of capillary constriction. Slade, Cotton and Lewis (24) saw the reaction well developed in cases of "irritable heart" and adopted the view that it was a capillary phenomenon.

Krogh (25) reported that scratching an area on the frog's tongue causes dilatation of capillaries and arterioles, usually over an area greater

than that which had been stimulated. He concluded that the reactions of capillaries could be independent of arterial pressure. The effect of electrical stimulation on arterioles was inconstant, and the effect of an electrical current on capillaries was very doubtful. He found that pure CO_2 caused dilatation of both arteries and capillaries. In general, all the substances tested by him gave the same effect on the capillary wall; relaxation of its powerful tone, which it acquired again after a period of several minutes. He was convinced that the spreading of the effects of stimuli was an axone reflex. He also found (26) that the skin capillaries of the frog react to mechanical and chemical stimuli independently of arteries, but to a less degree than those of the tongue, and that single capillaries undergo spontaneous changes in diameter.

Another proof of the independent contractility and dilatation of capillaries was also given by Dale and Laidlaw (27) and Dale and Richards (28). They pointed out that histamine when injected into the circulation of cats, caused dilatation of capillaries, together with constriction of aterioles. This was confirmed by Rich (29), Doi (37) and Hooker (30). Hooker also found that the injection of histamine destroyed the response of capillaries to electrical stimulation of the sympathetic nerve. Hooker described the post-mortem changes of capillaries of the cat's ear, showing the independent action of the capillaries. Such post-mortem changes of capillaries were also abolished by the injection of histamine (30).

Methods. In one series of the writer's experiments, the capillaries were excited by electricity. The same induction apparatus was employed in the experiments on frogs, bats and men. It consisted of a primary coil of 680 windings, and a secondary coil of 5000 windings. One, or in some cases, two dry cells were connected in the primary circuit. A different coil was used in the experiments on tadpoles and fish.

When the direct current was used it was obtained from one or two dry cells, or from a generator giving 60 volts. When the latter was used, the strength of the current was modified by shunting a part of it through a rheostat. A milameter in the circuit measured the strength of the current, and a commutator permitted the direction to be changed.

In order to localize the stimulations, unipolar methods were employed. When the induction current was used, the indifferent electrodes was connected to one pole of the induction apparatus, the other pole being left free, as a rule. The active pole was in many of the experiments a fine platinum wire, soldered to one end of a brass wire, the other end of which was either left free, or in case a stronger current was needed, con-

nected to an insulated sheet of metal, or to the observer's body, to enlarge the surface to be charged.

When the direct current was employed, some of the experiments were made with polarizable, and some with non-polarizable electrodes. When the non-polarizable electrodes were employed, the indifferent pole was a Harvard boot electrode resting on moist gauze connected with the body of the animal, and the exciting pole was a fine pointed camel's hair brush, which was fastened into one end of a glass tube by a plug of kaolin, moistened with physiological salt solution. The tube contained a solution of zinc sulphate, and an amalgamated zinc rod, which was connected by a wire with the battery. In some cases the camel's hair brush was tied to the tip of a Harvard boot electrode.

In order that the exciting electrode might be adjusted accurately, and so as to exert the least possible pressure on the capillary to be excited, it was supported by a binding post, which was clamped at the extremity of a straight rod, which was fastened to the vertical arm of an L rod, which was fastened by a clamp to a short rod projecting downward from a plate screwed to a microscope, in the ordinary position of an objective. By sliding the microscope on the table, by lateral movements of the horizontal rod, and by use of the adjustment screws of the microscope, the active pole could be brought in contact with the skin over the desired capillary, without exerting undue pressure.

Experiments on the web of frogs. The frog was pithed and the skull cavity plugged with a match to prevent loss of blood. The animal rested on a frog board. One web was spread out and fastened with threads tied to the tips of two toes. Beneath the web there was a hole in the board to let the light come through. The web was below the heart level. The spinal reflex effects which might result from excitation, on the vessels in general, as well as on the vessels of the part studied, can be avoided by the section of the sciatic nerve. This was done in many of the experiments. It had the advantage of also preventing reflex movements of the limb when the excitation was given. It was recognized, of course, that it would cause vasodilatation and subject the capillaries to an unusually high blood pressure. This was, however, regarded as an advantage. If in spite of the high blood pressure, a capillary could contract when excited, the result would be more striking.

In addition to the effect of cutting the nerve, the condition of the web, too dry or too wet, the spreading of the current, the mechanical influence of the exciting pole, pressure upon the leg, stretching of the web and other influence might become sources of error. When the active pole is

fine enough and is adjusted so as to barely touch the skin over a single capillary, when the current is very weak, and the web just a little moist, and when other conditions are suitable, the sources of error appear to be under control.

Effect of a tetanizing induced current. One pole of the secondary coil of the induction apparatus was connected to a copper plate, the indifferent electrode, and the other pole was left free. The frog rested on a pad of wet gauze which was on the copper plate.

An eyepiece micrometer was employed, not only for measurement, but also for locating the stimulated point of one capillary easily and definitely. The active pole, the fine platinum wire was screwed down slowly until it just touched the skin exactly over a single capillary. In order to be sure that the result was not caused by a mechanical effect, the writer waited ten minutes before applying the current. Then the key was closed for about half a second. When the unipolar current was weak, there was no visible effect. If the current was strengthened, by enlarging the surface to be charged, the circulation of the stimulated capillary was seen to slow. A still stronger current was obtained by connecting the other pole of the secondary coil to the ground, and this strong excitation stopped the blood flow in the stimulated capillary. At that time the circulation in other vessels in the field was normal. Finally, the blood flow in the excited capillary recovered. Many experinents were made, and others were called in to verify the observations. Some of the results may be illustrated in fig. 1.

Capillary A was stimulated at the point a; the automatic interrupter was started by closing the key for half a second. The local flow of the capillary A gradually slowed, then ceased, while the blood flow in B and other capillaries continued as usual. Eight minutes later the circulation in A returned.

In a similar way capillary B was excited. B was stimulated with the same strength of current but for a longer time.

After 3 seconds the circulation in B began to slow.

After 18 seconds the circulation in B stopped.

After 9 minutes the circulation in B recovered.

As has been said, that effect was strictly localized. For instance, when the circulation in the upper part of B was stopped there were still corpuscles coming from the capillary F and flowing toward G, while above the stimulated point b, which was contracted and so blocked without the blood being coagulated, a few corpuscles oscillated between h and b and went back and away through A.

Effect of infrequent induction shocks. The arrangement of the apparatus, and the method of excitation, were the same as before, except that the battery and key were connected with different posts of the primary coil so that separate induction shocks could be given.

The results of the tests illustrated in figure 2 were as follows:

Capillary A was stimulated at the point a. Tapping the key three times during 5 seconds did not cause a visible effect.

Capillary A was excited at a by twelve makes and breaks during ten seconds.

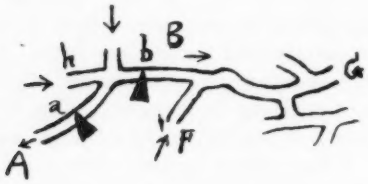

Fig. 1

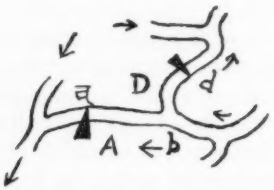

Fig. 2

Fig. 1. Capillaries A, B, were stimulated one after another. Arrows show the direction of the circulation. Triangles show the points where the active pole touched the skin over the capillary.

Fig. 2. Capillaries A and D were stimulated one after the other with infrequent induction shocks. Triangles show the points where the excitations were applied to the skin over this capillary.

After 5 seconds flow in A was slowed, but was normal in D.

After 12 seconds flow in A stopped.

After 16 seconds flow in A recovered.

After 2 minutes flow in A ceased again, but was normal in D.

After 3 minutes flow in A recovered again.

After 5 minutes flow in A stopped again.

After 10 minutes flow in A recovered again.

Capillary D was stimulated at d. Single make and break did not produce effect. Fifteen makes and breaks during 5 seconds gave the following:

After 4 seconds the flow slowed.

After 10 seconds the flow stopped, but was normal in A.

After 20 seconds the flow in D recovered.

After 1 minute the flow in D ceased again.

After $2\frac{1}{2}$ minutes the flow in D recovered again.

The repeated cessation and recovery of the local circulation suggested a rhythmical response of the stimulated capillary. When *A* was excited, one or two corpuscles passed the point *b* several times, but could not get through *a*. They showed to-and-fro movements between *a* and *b*, then went back through *D*. Apparently in such a case the constriction was localized at the middle of the excited capillary, at the point *a*, and the stopping of the flow could not be due to a constriction of the arteriole, because there was still movement of corpuscles between *a* and *b*, as well as through *D*, which was supplied from the same arteriole.

Repeated responses of capillaries have been described by others. In this case the nerve had been cut, so this must be regarded as a peripheral phenomenon. Looking at an unstimulated area, sometimes one could see the cessation of blood flow in one then in other capillaries. In this particular case, however, the repeated cessation and recovery were seen in the stimulated capillary, but not even in the neighboring ones supplied from the same arteriole.

Excitation by galvanic current. Two dry cells were used. The indifferent electrode was connected with the anode, the stimulating electrode with the cathode. When several excitations were given, the key was closed for a short time, opened and immediately closed again for a second period, and so on. The following effects were noted, the time of appearance of effects being estimated from the last closure of the key:

Capillary *A*. One excitation given during 1 second:
No effect.
Capillary *B*. Eight excitations given during 5 seconds:
Flow slowed after 7 seconds.
Flow stopped after 22 seconds.
Flow recovered after 24 seconds.
Capillary *C*. Ten excitations during 4 seconds:
Flow slowed after 8 seconds.
Flow stopped after 20 seconds.
Flow recovered after 3 minutes.

The current was reversed, so that the active pole became the anode, and another series of experiments was made. A part of the series may be tabulated as follows:

Capillary *A*. Eight excitations during 4 seconds:
Flow was slowed after 7 seconds.
Flow stopped after 15 seconds.
Flow began again after 2 minutes.
Flow normal after 3 minutes.

Capillary *B*. Twelve excitations during 5 seconds:
Flow was slowed after 10 seconds.
Flow stopped after 15 seconds.
Capillary *C*. Fourteen excitations during 7 seconds:
Flow was slowed after 10 seconds.
Flow stopped after 30 seconds.
Flow began again after 32 seconds.
Flow was normal after 39 seconds.
Capillary *D*. Twenty excitations during 8 seconds:
Flow was slowed after 15 seconds.
Flow stopped after 3 minutes.
Flow began again after 5 minutes, 25 seconds.
Flow stopped again after 6 minutes.
Flow started again after 6 minutes, 30 seconds.

In the case of *A* and *B*, the stimulated capillary was packed with stagnated corpuscles. In the case of *D*, a comparatively frequent excitation aroused repeated response.

In the following experiments non-polarizable electrodes were used. The active pole was the cathode. The results of a part of the tests were as follows:

Capillary *N*. With one cell, ten excitations in 1 minute:
No effect.
Capillary *O*. With two cells, four excitations in 1 minute:
Flow slowed after 1 second.
Flow became normal after 23 seconds.
Capillary *P*. With two cells, four excitations during 1 minute, 30 seconds:
Flow stopped immediately.
Flow started after 86 seconds.
Flow normal after 4 minutes.
Capillary *R*. With two cells, four excitations during 80 seconds:
Flow stopped immediately.
Flow started after 162 seconds.
Flow normal after 4 minutes, 30 seconds.
Capillary *S*. With two cells, four excitations during 80 seconds:
Flow stopped immediately.
Flow started after 162 seconds.
Flow normal after 4 minutes, 30 seconds.

In the case of *P*, a very localized constriction was observed. With non-polarizable electrodes at least two dry cells were needed. Experi-

ments seemed to show that a longer duration of excitation tended to prolong the cessation of corpuscular flow in stimulated capillary.

Experiments on the fin expansion of tadpoles. These, with the experiments on fish were made at the Marine Biological Laboratory in Woods Hole.

Method. A somewhat different method was employed from that which was used in the experiments on frogs. An upright chamber was made, such as Professor E. R. Clark (27) found useful in studying lymphatics. The back of the chamber consisted of a glass slide, 39 × 75 mm; the front, of cover glass; the sides and bottom, of three narrow strips composed of two thicknesses of window glass; and all of these were cemented in place with canada balsam, the top being left open. The floor of the chamber was coated with a film of paraffin.

In experiments the slide was clamped to the stage of a microscope, which was arranged with the tube horizontal. The tadpole, to be examined, was anesthetized in a dish of chloretone solution, of a strength of 1:3000, or 1:6000, and then transferred carefully from the dish to the cell by means of a medicine dropper.

The tadpole was brought carefully to the side of the chamber next to the cover glass by means of a blunt needle. A narrow piece of cover glass was set on the floor of the chamber, leaning against the cover glass which formed the front wall, and was brought against the tail of the tadpole by pressing gently with the blunt needle. Since the narrow piece of cover glass came in contact with the thick, central, muscular portion of the tail, pressure on the fin expansion of the tail was avoided.

By using the upright chamber, the tadpole could remain in its normal position, so experiments of long duration were possible, without the complications which might result from an abnormal position. The apparatus for adjusting the active electrode was the same as described already, except that it was arranged with the tube of the supporting microscope in the horizontal position. In this way the stimulating electrode could be moved backwards and forwards.

The indifferent electrode was brought in contact with the fluid near the body of the tadpole, the fluid itself really becoming the indifferent pole. The stimulating electrode reached the fin expansion of the tail from the right corner of the chamber. A tetanizing current was employed.

In a number of cases, the blood stream in the stimulated capillary became slow, but did not stop. In a few cases, however, the circulation stopped at the place where the capillary was excited. In four cases,

changes of shape of corpuslces passing the stimulated part of the capillary were clearly observed. It was possible with an upright chamber so constructed, to make experiments with the highest powers of the microscope, even with the oil immersion. The endothelial cells of the capillary wall could be well observed. Sometimes after the application of the stimuli, the stimulated capillary disappeared from view under the low power, but was still to be seen with the highest power and looked narrowed.

Experiments on the caudal fin of fish. When the writer was working at the Marine Biological Laboratory in Wood's Hole, he found that some of the fish could be used to advantage for studying the response of capillaries to excitation.

The technique used was very simple. The unipolar method of excitation was employed. The fish was placed on a glass plate. A mass of moistened filter paper acted as the indifferent electrode. The active pole, the holding and adjusting apparatus were the same as described already. The fish was usually anesthetized with chloretone. Unanesthetized fish were also used in a few experiments of short durations. The fish were kept moistened.

Experiments were made on the caudal fin of Fundulus heteroclitus and Cyprinodon variegatus, and also on the skin capillaries of the abdomen of the small toad fish. The capillaries actively responded to the tetanizing current and to a series of separate induction shocks, or to excitations with the direct current.

The corpuscular flow in the stimulated capillary became slow and then stopped. The relation between the duration of the excitation, and that of cessation of circulation in the stimulated capillary was irreguiar. With the same duration of excitation, the results were not exactly the same. The response of the excited capillary, however, was always strictly localized.

Experiments on the wing of bat. Among warm-blooded animals, bats possess the advantage for our purpose of having transparent wings and of belonging to the mammals. The bats were hibernating, and were kept in a cold room. When used they were aroused from their sleep by the handling. They were etherized, and the wing to be examined was placed so that it was below the heart level.

The bat was placed on his back, on a suitable holding board. The left wing, which was to be studied, being stretched across a hole in the board. The stimulating electrode was brought to touch the wing either from above or below. The unipoplar method, as described for frogs, was used.

Influences affecting the resistance to the flow, and spreading of the current had to be considered. It was found that the bat's wing which is coated with keratin substance has greater resistance than the frog's web. A strength of current that would alter the flow of the blood through the frog's web failed to be effective in the case of the bat's wing. When the current was strong, there was an effect, but there might be a tendency to injure the tissue, and so too strong currents had to be avoided. When the bat's wing was moistened with water, it appeared to gather together in fine droplets, which, if sufficiently numerous, formed a sheet of water. If the active pole touched a layer of water, the current spread, and was not so effective as when the current was condensed at a very fine point.

In as much as disappearance of blood from a capillary was assumed to be evidence that it was contracting, the level of the capillaries with respect to the heart was important. When the vessels are above the heart level, constriction of the arteriole may empty them. Hooker (30) made the following experiment: A cat was placed on an animal holder; the capillaries of the ear were about 3 cm. below the heart level. The arterial supply to the area under observation was mechanically shut off, so that there was no movement of red cells in the capillaries, but the latter vessels did not empty. As the part to be stimulated in my experiments was always below the heart level, constriction of the arteriole alone could not empty the capillary, and this error of experimental procedure was certainly avoided.

At first the picture was not so clear as in the frog's web, but with practice one could overcome the early observational difficulties. Instead of giving a net-like appearance, as in the case of a frog's web, the capillaries of the bat's wing are longer, and look like the small branches of a tree. In some cases the presence of capillaries was indicated only by a stream of corpuscles; while in other cases a faint line representing the capillary was seen though there were no corpuscles then present. To be sure that the vessels to be excited were not arterioles or venules, the smallest capillaries were chosen for excitation.

The tetanizing current did not cause any visible contraction of the bat's capillary, when the strength was enough to excite the capillary of the frog's web. Even when one pole of the secondary coil was connected with the active electrode there appeared, except in one case, no change in the microscopic field.

Excitations with infrequent induction shocks. When one pole of the secondary coil was connected to the indifferent pole, while the other

pole was left free, and the brass wire to which the stimulating electrode was soldered was also free, no result was obtained. Even when the active pole was connected to the operator's body, closing and opening the key once did not arouse the contraction of the bat's capillary. When, however, the key was opened and closed twice at 3-second intervals, a distinct slowing of the circulation was observed. The following is a typical experiment (fig. 3):

Capillary *A*. Stimulated at point *a* by closing and opening the key three times at intervals of 3 seconds.

After 1 second flow in *A* slowed.

After 145 seconds the corpuscles came through in single file slowly, but continuously.

After 180 seconds corpuscles came through at intervals.

After 223 seconds the blood flow in capillary *A* stopped but was visible in neighboring capillaries.

Fig. 3

Fig. 4

Fig. 3. Capillaries *A* and *B* were stimulated one after another, at points *a* and *b*.

Fig. 4. Showing alteration in form of corpuscles when passing the excited region, about the middle of capillary *P*.

After 264 seconds the flow was restored, corpuscles coming one by one or in small groups.

Capillary *B*. This was stimulated at the point *b* by closing and opening the key three times at 3-second intervals.

After 134 seconds corpuscles came through at intervals.

After 185 seconds, blood flow in *B* reversed.

After 203 seconds, flow in *B* stopped, but was visible in neighboring capillaries.

After 275 seconds, one corpuscle moved to and fro between *b* and *c*, and then forced its way through the stimulated point *b*; then four or five corpuscles followed, one by one; then the circulation stopped again; then four or five corpuscles forced their way through *b*.

The most constricted part probably was the point *b*, where the current was condensed. This was shown by the to and fro movement of the corpuscles between *b* and *c*, and by the fact that the corpuscles could pass the point *b* only with difficulty. As soon as they passed *b* they

went through freely. This observation also showed that the most apparent response was limited to the part stimulated, about the middle of the capillary *B*.

Excitation by the direct current. The electric current was supplied from a dynamo giving sixty volts. A mercury key permitted the current to be sent through a rheostat, where it was shunted, so that any desired amount could go through a milameter, and then by way of a commutator, which allowed the direction of the current to be changed, to the indifferent pole, which was the cathode, or in case the current was reversed, the anode. The current was closed for a brief time, then opened and closed for a brief interval again, so that a series of excitations was given.

In the following tests the active pole was the cathode. A few of the tests are recorded below.

Capillary *L*. Two closures of the key during 10 seconds.

No effect.

Capillary *M*. Four closures in 1 minute.

After 10 seconds, flow was slowed.

After 20 seconds, flow was normal again.

Capillary *N*. Four closures in 3 minutes.

After 2 seconds, flow stowed.

After 5 seconds, flow stopped.

After 69 seconds, corpuscles oscillated.

After 135 seconds, flow normal.

Capillary *P*. Four closures in 3 minutes.

After 11 seconds, flow slowed. Corpuscles changed their shape when passing through the middle of the capillary.

After 16 seconds, flow stopped.

After 82 seconds, corpuscles oscillated.

After 130 seconds, flow normal.

Capillary *R*. Four closures in 3 minutes.

After 4 seconds, flow stopped.

After 80 seconds, corpuscles oscillated.

After 310 seconds, flow normal.

In this experiment too, the corpuscles were seen to alter their form in passing through the constricted area.

In the case of *L*, in which the current was closed and opened twice during 10 seconds, no effect was seen. In the case of *M*, in which the current was made and broken four times during the course of one minute, the flow was slowed. In the case of *N* in which four closures were made

during 3 minutes, the circulation in the stimulated capillary was slowed and stopped. According to the general law of polar excitation of nerve and muscle, as recognized by Pflüger (7) and Von Bezold (34), the effect of stimulation appears at the cathode upon making, and at the anode upon breaking the current. The present experiments tend to show that the duration is important. This is, however, not striking if one considers the cases given by Kühne (35), and Verworn (36) for they show that the constant current stimulates throughout the duration. The experiments on *N*, *P* and *R*, when the current was given in the same manner, showed different effects.

The experiments on *P* and *R*, were observed under high magnification, and the shape of the corpuscles were seen to be changed as they passed through the stimulated part of the capillary. Corpuscles, when they were passing the excited region, about the middle of capillary *P*, for example, one by one altered their form, and acquired a comma shape (see fig. 4). This suggested that the capillary was partially blocked by a very localized narrowing of the lumen, as if there was a protoplasmic protrusion of the endothelial cell. The protrusion, although it was not seen as such, forced the corpuscle to show a dent, which moved from one end of the corpuscle toward the other end, as the cell was passing the part.

In the case of *R* the corpuscle showed various changes when going through the affected part of a single capillary. It began to penetrate the partially blocked region with a narrowed end. When it had passed half way through the constricted area, the portion that had passed resumed its original shape, so that the corpuscle had a dumb-bell form. Then as the corpuscle continued to pass through, the hind end became narrowed. After the whole cell had passed the protoplasmic protuberance of the capillary wall, the corpuscle resumed its ordinary form.

In the case of *P* and of *R*, a localized contraction was observed.

Stimulation with non-polarizable electrodes. In this experiment the indifferent pole was a boot-electrode which rested on the moist cloth on which the bat lay. The active pole was a sharp brush, fastened to the end of a piece of glass tubing by a plug of kaolin. It is unnecessary to relate the results in detail; they were similar in character to those obtained when a polarizable electrode was used as the active pole.

Experiments on human capillaries. Method. The capillary loops of the human skin may be directly observed by following Lombard's method. This method consists of moistening the skin with a drop of glycerine or transparent oil, and observing by a strong light with a

microscope. A Zeiss binocular microscope has the advantage of taking any desired position, and affording a large working distance. The ordinary microscope is also of great service. The latter in practice was found more convenient for the localization of a particular capillary. When capillaries of the thumb are to be examined, one may turn the front edge of the stage of the microscope toward the finger. Too high powers have the disadvantage of losing definition and depth of focus. An artificial illumination is better than the day light, because the former can be more readily adjusted.

The writer stimulated and observed capillaries of his own finger, or of the back of his hand, either radial or ulnar side, or the skin of the fore arm. The fore arm rested on a wooden support, at one end of which there was a piece of wood which could be moved up and down to fit the palm. When the finger was examined, the arm support was used and the finger rested on the microscope stage. There should be no movement or tremor of the part to be examined, and no pressure should be brought on the blood vessels or nerves of the arm. As Danzer and Hooker (12) have pointed out, the part to be studied should be clean and free from moisture. The skin should be scrubbed with soap and water, then thoroughly dried. If there is moisture between the skin and oil, the field is less clear.

In case electrical stimulation was to be employed, a drop of glycerine instead of oil should be used. Some control experiments were made to test the conductivity of glycerine and oils. In the case of oil, the galvanometer oscillated but little, unless the electrode pressed heavily on the skin. As soon as the electrode touched a drop of glycerine the conductivity as indicated by the galvanometer was apparent.

The appearance of the superficial capillaries of the human skin. If Lombard's method be followed the epithelium is not seen, but the underlying corium with its papillae is observed. As the strong light penetrates a short distance into the corium itself, its connective tissue is sufficiently transparent to show the capillaries of the papillae. The condition of the skin determines the picture observed. Sometimes one sees only a few, and at other times many capillary loops against a pale yellow background. Sometimes the background has a rose tint, the red being unevenly distributed. Some of the loops are twisted on themselves, some capillaries look like simple arcs. While the loops of the ulnar side of the back of the hand are short, long loops are seen on the radial side.

Lombard observed a capillary loop communicating wtih a superficial veinlet, and that when pressure was brought to bear on the skin over the capillary loop, the side toward the vein emptied before the other side of the loop, which was undoubtedly in communication with an arteriole which was deeper down and not visible. The veins were more superficial, and the arterioles lay deeper down in the corium and could not be seen. The writer failed to see such venules in his skin, and the flow of the blood was too rapid to permit the direction to be observed, unless it was slowed by pressure, or by the constriction of the capillary caused by stimulation.

Sources of error in studying human capillaries. The movement and tremor of the field under observation might give a wrong impression. Care must be taken to distinguish the disappearance of a capillary caused by the stimulation, from that produced by loss of focus caused by movement of the part. After working several days continuously, one could locate and recognize a definite capillary more easily, and could tell the difference according to the following method. The capillary to be stimulated was in the same plane with at least some of the other loops. Before stimulation, control experiments should be made. Loops in the same plane would disappear, and reappear at the same time by changing the focus. If the disappearance is limited to one particular capillary while neighboring loops of the same plane are still visible, then it must result from stimulation.

The phenomenon of diasppearance was not the only kind of response caused by stimulation. Sometimes after stimulation the loop was still visible, but slow circulation resulted. Ordinary capillaries looked like continuous red threads, arcs, or loops, and no indication of corpuscular flow was observed, as the circulation was too quick to show individual corpuscles. After the application of stimulation, the red cells were seen streaming slowly through the capillary, and the flow of corpuscles was evident. Danzer and Hooker (12) have shown that the corpuscular flow was seen under pressure of 18 to 27 mm. Hg. In this connection care must be taken to avoid any pressure. It was not impossible to avoid pressure as the active pole was either a very slender wire or a delicate camel's hair brush and fine adjustments were provided. In as much as Lombard described a case of vasodilatation caused by pressure on a nerve, care had to be taken that this did not occur.

Danzer and Hooker found that it frequently happened that when one obtained a focus of the capillary field, one or more of the capillaries stood out conspicuously large. They stated that such capillaries

represented vessels in which the corpuscular flow had stagnated, and corpuscles were thickly packed together. Even a high extra-capillary pressure did not change the appearance of such vessels. Bearing this in mind, one would assume that the lack of visible change after stimulation in some cases might be due to the condition of the capillary.

The secretion of the skin might cause trouble. Clean and dry skin was required for obtaining a clear picture. When sweat intervened at the coil-skin boundary, the intimate penetration of the oil was interfered with, and the clarity of the field was lost. Indeed, sometimes, during the long experiments, the field became less clear, and one might mistake that the capillary under observation disappeared. This is, however, easy to differentiate. If it is due to the secretion of the skin, the picture will not become clear again unless the skin is cleaned once more. If it is a real disappearance, the capillary will reappear.

Excitations with a tetanizing current. The induction apparatus, dry cell, the electrodes and the key were the same as those employed in studying the bat's capillaries. The copper plate, which acted as the indifferent pole, was placed on a wet pad on the arm. As the writer worked on his own capillaries it was found to be more convenient to make the observations on the left hand. Capillaries stimulated with the tetanizing current did not show visible change.

Excitations with infrequent induction shocks. The part to be examined was about 12 cm. below the second intercostal space. The capillaries in the skin at the base of the nail of the ring finger, at the ulnar side, and at the radial side of the back of the hand, and in the skin at the lower part of the fore arm, were studied. The skin of the fore arm looked softer than that of the back of the hand, but the tremor caused by the pulse was greater.

A capillary on the back of the hand was chosen and watched for a while, and the condition of this capillary, and of the neighboring ones was noted. The active pole, the platinum wire, was adjusted to gently touch a point of the skin just over the capillary to be stimulated. Enough time was allowed to let the mechanical influence pass off, if there was any.

Single making and breaking shocks did not bring any visible response. The same capillary which did not respond to single shocks, was affected by a summating series. Before excitation, capillaries appeared as pink threads, no individual corpuscles being seen. The key was then closed and opened four times at an interval of two seconds each. The following was noted:

After 3 seconds, minute bodies, the red corpuscles, were seen moving along the stimulated loop, but no change in neighboring capillaries.

After 10 seconds, the minute bodies passed one or two at a time.

After 15 seconds, movements of bright minute bodies were followed by groups of corpuscles, the observations of the latter being facilitated by the former.

After 30 seconds, normal again.

In certain cases when the flow of the corpuscles was not seen, the part of a capillary stimulated merely appeared less distinct. For example, the writer in some cases saw at one point of the stimulated capillary a constriction, which broke the continuous appearance of the red loop.

In other cases, the constriction did not break the continuous outline, but before the application of the stimulation, the red color of the whole loop looked almost even, after the excitation, the color became uneven.

Evidently some change took place at the stimulated part of the capillary. The slowing of circulation might have been due to a localized constriction. Concerning the localized change of appearance of the red loop, one might think that the number of red cells passing the stimulated part might have been decreased as a result of increased friction.

The following experiments were made on the skin at the base of the nail of the ring finger of the left hand, the finger being 13 cm. below the second intercostal space. Groups of make and break induction shocks were used. The effects were confined to the capillaries which were excited.

Capillary *a* was excited eight times in 15 seconds. Circulation of capillary was slowed.

Capillary *b* was excited fourteen times in 13 seconds. Red color of the loop became uneven.

Capillary *c* was excited ten times in 18 seconds. Circulation of the capillary was slowed after 3 seconds.

Capillary *d* was excited ten times in 18 seconds. Blood flow of the capillary *d* was slowed after 10 seconds.

Capillary *d* was excited ten times in 15 seconds. The continuous appearance of the red loop was broken near the arc part.

Capillary *f* was excited twelve times in 15 seconds. No visible response.

Capillary *g* was excited twelve times in 15 seconds. Blood flow of the loop was slowed.

Effects of direct current. The electric current was supplied by a dynamo generating six volts. The stimulating pole was the cathode. As the active pole was polarizable the passage of the current caused the liberation of hydrogen gas, at this pole, often as fine bubbles which tended to obscure the field. The gas bubbles did not last very long, and experience made the field appear clearer. Nevertheless the latent period could not be accurately estimated.

After the application of a series of excitations, in some cases, the circulation of the stimulated capillary was slowed; in other cases, the capillary became less red at the arc of the loop. In still other cases, the stimulated capillary disappeared and reappeared.

Sometimes an appropriate stimulation caused a single capillary or a part of the capillary to disappear and reappear again and again. The duration was irregular, generally that of the disappearance was shorter. The disappearance was not due to constriction in the arterioles, which might have prevented the blood from reaching the vessels distal to the constriction, for the part under direct observation was below the heart level and the phenomenon was localized to the capillary that was excited, neighboring capillaries remaining unchanged. This would be impossible if the response was not given by the capillary itself.

In the following experiments non-polarizable electrodes were used. The stimulating electrode was the cathode. Instead of glycerine, a drop of 0.1 per cent or 0.2 per cent salt solution was put on the part to be examined as the latter conducted the current better. The brush was washed and moistened with the same solution. When one is accustomed to seeing the capillaries, they can be observed if the skin has softened by glycerine and oil for several days, even when a drop of water or very dilute salt solution is placed on the skin after oil has been thoroughly removed from the surface.

Sources of error. Under a drop of the dilute salt solution capillaries looked smaller and less distinct than when glycerine was employed. The skin became dry easily, and even before this, a concentration of salt solution may interfere with the picture. Sometimes the clarity of the field was lost entirely or partially. A partial loss of clarity might shade the capillary under observation. This must not be mistaken for the disappearance of the vessel. On the other hand a positive reaction might be mistaken to be negative when the phenomenon could not be clearly observed. In spite of the difficulties, the use of non-polarizable electrodes, because eliminating the gas bubbles, was advantageous. The difficulties were overcome by cleaning the field diligently, by renewing the drop of salt solution, and by untiring work.

In a number of experiments individual capillaries were stimulated with the same length of period but various numbers of excitations and the observed reactions were irregular.

The following experiments were made on the skin at the base of the nail of the ring finger of the left hand, the finger being 130 mm. below the second intercostal space. Non-polarizable electrodes were used.

The above experiments were made with the same length of period and the same number of excitations. The results, however, were different in various cases. One would think that conditions of various capillaries, or of one capillary at different moments, were not the same, which might determine the reaction observed. (Table 1.)

DISCUSSION. *Is the absence of muscular coats unfavorable to the view that the blood capillaries have the power of active response to stimuli?* As the blood capillaries consist of endothelial cells, which are not as highly differentiated as muscular tissue, the old view assumes that

TABLE 1

CAPILLARY	NUMBER OF CLOSURES	PERIOD OF EXCITATION	OBSERVATIONS
		minutes	
R	8	3	Flow in capillary *R* slowed
R	8	3	No visible reaction
R	8	3	*R* disappeared and reappeared
R	8	3	Blood flow in the single capillary slowed
Z	8	3	Capillary disappeared and reappeared

changes in caliber of capillaries is dependent on passive response to alterations of the pressure of the blood supplying them. There is, however, evidence to show that cells which are not as highly differentiated as muscle cells can respond actively to stimulation. The melanophore of Fundulus heteroclitus responds to adrenalin (31), and to other kinds of stimuli (32). The writer himself has observed changes of form of pigment cells in the skin of frogs and tadpoles.

If an amoeba be stimulated with an electric current, that side of the body of the amoeba which is directed toward the anode contracts (33), then a pseudopodium starts out somewhere on the side directed toward the cathode. There are many other examples of active contraction of very simple forms of protoplasm, and therefore, if in the absence of muscular coats, a protoplasmic unit, such as the amoeba, has the power of active response—changing its form and producing a protrusion,—why should not the property of contraction be possessed by the protoplasm of endothelial cells?

Is capillary constriction an active process? The old conception was that a narrowing of the capillary lumen is an elastic phenomenon, a passive process, which occurs when there is decreased blood pressure in the capillaries, which is caused by constriction of arterioles, or by a low general blood pressure. There is no doubt of the elasticity of capillaries, and that a lowering of internal pressure would tend to cause the lumen to narrow. This does not always take place, however. Roy and Brown (20), by tapping the abdomen of a frog, lowered its central blood pressure, and the pressure in the peripheral arterioles to nearly that of the atmosphere, but the capillaries did not change their size. They also mentioned that on amputating a frog's foot, the capillaries became very little narrower, although the pressure had been reduced to zero. When the writer cut a capillary net work—nearly free from arteries and veins—from an isolated scale of Fundulus, the capillaries did not change in caliber, although the blood pressure had been decreased. Dale and Laidlaw (4) proved that histamine causes a constriction of the arterioles and that this constriction did not prevent the widespread dilatation of capillaries. Heubner (22) has shown that the intravenous injection of the double chloride of gold and sodium produces a shock-like prostration. Microscopically, venules and capillaries of all the tissues were found to be dilated, but the small arteries were so strongly constricted as to occlude the lumina completely. It is evident that the narrowing of the lumen of the capillary does not depend simply on the pressure of the blood supplied to it, and that capillary response may be independent of arterial activity.

The writer, as has been stated in the description of his experiments, observed the constriction of a single capillary, even a part of a blood capillary, to take place without any recognizable change of immediately surrounding vessels. The change of the capillary wall was also indicated by the character of the flow of the blood corpuscles. Using a microscope giving a high magnification, a change of shape of corpuscles passing the stimulated part of a capillary was distinctly observed. The most suitable place for applying stimulation was the middle of a long capillary. With an appropriate stimulation, the arteriole, the venule, and parts of the capillary on both sides of the stimulated point remained unaltered. A corpuscle, when travelling the arteriole and a part of the capillary, showed its usual shape, but when it came to the excited part, it exhibited various forms, one after another (see fig. 4). When it reached the part of the same capillary beyond the excited region, it resumed its ordinary form.

The writer would like to emphasize that not only the capillaries of animals, but also of the human skin, can respond to adequate stimuli. In the case of human capillaries, as has been shown by his experiments, the reaction, such as a slowing of the corpuscular flow, a disappearance, or a change of appearance of the stimulated capillary, could not be due simply to a constriction of arterioles, because the response was usually very localized. It seems as if there is no doubt that the capillary constriction is an active response.

Is the capillary dilatation dependent simply on a rise of blood pressure? The writer believes that it is a change in the condition of the capillary wall itself that causes the dilatation, and that the capillaries are not enlarged or opened up simply by an internal pressure. As Krogh (1) has pointed out, the arterial pressure is unable to dilate capillaries to any appreciable degree, or to open them up when tonically constricted, while the venous pressure may be sufficient to fill them when relaxed in response to local stimulation. If the opening up of capillaries be merely a passive process, then those with least resistance would always have an easier chance. If this be the case, the blood would always flow through the same definite channels when the capillary pressure is low, and the distribution of oxygen and nutritive substances would be very unequal, the cells lying nearest to the open capillaries being well supplied, while those at the greatest distance were starved. He has also added that the pressure necessary to force muscle capillaries open when they are actively contracted is much higher than the normal arterial pressure.

The evidence that capillaries are not simply passively expanded by the increased intracapillary pressure, which results from an arteriole dilatation, or from a rise of general arterial pressure, comes from many sides. For instance, in Stricker's experiments, the nictitating membrane of a frog was removed from the body and therefore deprived of its blood supply, yet an evident dilatation of the capillaries could still take place. Roy and Brown (20) have shown that with a very low capillary pressure, capillaries remain dilated by the application of chloroform. The writer has applied chloroform to an isolated capillary net work of Fundulus, dilatation of capillaries in response to chloroform was still present, in spite of the fact that there must have been very little pressure either in the arterial or the venous sides. Again as pointed out by Roy and Brown (20) in the case of hyperemia, which follows temporary anemia, also, the dilated capillaries remain expanded, although by the "Klopfversuch" their internal pressure has been reduced to only a little above

zero. Moreover, Dale and Laidlaw (4) have shown that histamine causes a widespread dilatation of capillaries of cats, independent of its effect on arterioles, which are constricted. Krogh (25) has found that urethane causes dilatation of capillaries without affecting the arteries. He concludes that capillary dilatation is independent of blood pressure.

The writer has noted several cases of very localized dilatation. This was not only independent of arterial dilatation, but also independent of the condition of the rest of the same capillary. The point under observation was larger than the rest of the vessel, moreover, the blood stream passing through it became gradually slower, and at last that part became packed with corpuscles. If it was simply expanded passively by intracapillary pressure, why did not the pressure expand the rest of the capillary? The observations suggested that the reaction was associated with a vital response.

Are endothelial cells the agents of the active response of blood capillaries? Stricker (14), Roy and Brown (20), Dale and Laidlaw (4), Hooker (34) and Krogh (1) have the opinion that the endothelial cells are responsible for the capillary phenomena. On the other hand, Rouget (16) and Steinach and Kahn (21) had the view that certain perivascular elements caused the capillary constriction. As has been pointed out by Hooker (38), the vessels which they regarded as capillaries, were probably arterioles. Working with a microscope of very high power, the writer observed extremely clear pictures of the endothelial cells and their nuclei, of the capillaries of the fin expansion of tadpoles, but failed to see any such perivascular elements, yet these vessels contracted when locally excited.

There might be two possibilities: *a*, contraction of the endothelial cells of the type to narrow the lumen so that the blood would cease to enter; *b*, bulging of the endothelial cells, so that the lumen of the capillary would be encroached on enough to prevent the entrance of the blood. In the case of human capillaries, the vessels were not observed as such, but by their contained blood. In the case of the bat's wing, sometimes the outline of the capillary wall was shown, though at other times it was not seen even when the blood was flowing. In the latter case,when the blood ceased to enter the capillary, the indication of the presence of a capillary ceased.

The alteration of the shape of individual corpuscles, which the writer also observed, when they were passing, one by one, a stimulated part of a capillary (fig. 4) would suggest that the excitation might cause a bulging out of a part of the endothelial protoplasm to block the capillary

lumen partially; or that the phenomenon might be due to a contraction of the endothelial cells of such a type as to make the capillary lumen irregular. The experimental facts, therefore, tended to show that the endothelial cells were the agents of the active response of the blood capillary.

SUMMARY AND CONCLUSIONS

The unipolar method of electrical stimulation was employed to study the effect of direct, localized excitation on the blood capillaries of frogs, tadpoles, fish, bats, and men. Their active response was detected by observation with the microscope.

Induced as well as direct currents were employed. Single make and break shocks did not cause visible effects; a summating series was required to elicit a response.

Capillaries of the web of frogs and of the caudal fin of fish respond to a tetanizing current, and to a series of induction shocks. They also responded to the direct current, when a series of excitations was employed.

Capillaries of the bat's wing were seen to respond only once to a tetanizing current, but they reacted to separate induction shocks and to the direct current.

Capillaries of the human skin did not react to a tetanizing current, but responded to separate induction shocks. They reacted also to the direct current when a series of excitations was used.

Capillaries of the same tissue, stimulated in the same manner, gave various effects concerning the latent period, the duration of contraction, of slowing of flow, stopping of flow and disappearance of capillaries.

When the capillaries of the bat's wing were studied under a high power, a change of shape of individual corpuscles passing through a stimulated part was observed.

That the blood capillaries respond to stimulation actively, that both capilo-constriction and capilo-dilatation are active responses, and that the capillary endothelium is responsible for the phenomenon have been suggested by experimental results. The fact that the reaction to stimulation was strictly localized, would seem to justify the conclusion that the result was an active response of the wall of the capillary.

The writer wishes to express thanks to Prof. W. P. Lombard for his various suggestions, to Dr. L. V. Heilbrunn for his suggestions, especially when working in Woods Hole, to Dr. O. M. Cope for his help in in-

jecting cocain during another series of experiments on human capillaries, to Dr. W. C. Curtis of the University of Missouri for securing bats, and to Dr. F. R. Lillie, Director of the Marine Biological Laboratory, for the privilege of carrying a part of the work there.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

IV. The Nature of Muscular Contraction in Auricular Fibrillation and Flutter

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Received for publication July 17, 1922

Functionally, the auricles may be regarded as continuous units or fractions of muscle syncytium, each capable, when excited, of responding by a tiny "fractionate contraction" which lasts on an average 0.047 to 0.06 second (1), (2). The extent, rapidity and duration of the contraction process which any strip of auricular tissue undergoes will therefore be determined *a*, by the number of muscle fractions excited; *b*, the vigor of each fractionate contraction, and *c*, the relative number of fractions which are contracting or relaxing at the same time. The latter will depend on the rate at which the impulse travels as well as on the order in which the units are excited.

Normally, when impulses spread regularly across the auricle from the sinus node, the spread of excitation is such that the more proximal fractions receive their impulses before those which are more distally situated. Consequently, the former not only begin to contract and relax earlier, but the mechanical effects are such that the more proximal fractions are actually in the process of relaxing when the more distal ones are still shortening (1). In consequence, the systolic auricular shortening accurately recorded from auricular points about 20 mm. apart, shows distinctive changes in gradient and comes to an actual stop when a balance between contracting and relaxing fractions in this section obtains. As such a balance can only be a momentary affair, the myograms come to a rounded peak (fig. 1) and do not present any plateau effect such as Lewis, Feil and Stroud (2) have recently emphasized.

Many questions at once suggest themselves as to how the mechanism of contraction is modified when impulses no longer reach the auricular

fractions in normal fashion. Do coördinated contractions obtain in flutter and fibrillation so that we may truly speak of a systole and diastole in both conditions? If so, in what ways do they resemble or differ from the normal? Are the contractions regular or irregular in amplitude, steepness and duration? Is there any definite relation between the auricular contractions and ventricular systoles or do auricular and ventricular events occur in haphazard fashion? Finally, are the changes in contraction entirely referable to irregular or abnormal summations of fractionate contractions, or are these contractions themselves fundamentally different?

In an experimental investigation of auricular fibrillation carried out in 1917, Niles and I (3) hoped to throw some light on these questions. Finding, however, that our stock of facts did not warrant any detailed analysis, we were forced to content ourselves with the demonstration *a*, that the majority of waves recorded from the auricle in fibrillation do not represent true contraction phenomena, and *b*, that the majority of diastolic pressure waves in the auricle and large veins are not caused by auricular contractions.

Since that time, a large number of additional tracings taken during auricular flutter and fibrillation has accumulated. In some of these, flutter or fibrillation had been purposely induced by electrical stimulation, in others it supervened accidentally during the course of other experiments.

In view of the fact that the recent investigations of Lewis and his associates (4) have altered our conception of the nature of these disturbances, and have made it possible also to recognize more clearly the precise condition with which we are dealing, it seems desirable to see whether the previously unintelligible hieroglyphics of optical tracings might not be deciphered by the aid of our newer knowledge, and thus further our understanding of the contraction phenomena in fibrillation and flutter.

THE RECOGNITION OF FLUTTER AND FIBRILLATION IN EXPERIMENTAL WORK. *Definitions.* It is desirable to catalogue the conditions studied as nearly as possible in accord with the conceptions recently advanced by Lewis and his co-workers (4). According to these investigators, flutter is defined as a condition in which impulses travel at regular but reduced rates over definite anatomical rings, from which centrifugal waves are thrown off to the remainder of the auricular tissue, exciting each successive fraction of outlying muscle in an orderly and regular fashion. *Flutter* becomes *impure* when more or less irregular blocks appear

locally in the course of the centrifugal impulses alone so that they must pass through sinuous courses. When a similar sinuosity of the central excitation circuit develops and, in consequence, the centrifugal waves are thrown off to the peripheral portions of the auricle at irregular intervals—auricular fibrillation results.

The differentiating of flutter and fibrillation by direct auricular leads. In a considerable number of experiments, the mechanical contractions of the auricle between two points varying from 6 to 24 mm. were related to intrinsic deflections also recorded from these points. Consequently, it is desirable to review the criteria by which different forms of irregularity may be differentiated by the aid of intrinsic deflections derived from a single pair of auricular leads. The records published by Lewis and his associates (4) indicate that in flutter the intrinsic deflections are very regular in spacing and contour, while the ventricular deflections (if indicated) usually occur in definite relation to the auricular excitation. The curve published in figure 5, segment B in a former article with Niles (3), is an example and was at the time correctly interpreted. Occasionally I have found, however, that although the intrinsic deflections are perfectly regular, the incidence of the ventricular deflections is not quite so orderly (cf. fig. 2). The simpler types of impure flutter are also readily distinguished. The deflections are similar in contour but differ in that slight variations in spacing obtain. If a flutter is somewhat more complicated, minor variations in form are apparent, indicating that the spread of the wave through the area examined has altered. Frank instances of auricular fibrillation in which the rate is high are readily distinguished by the total lack of uniformity either in contour and spacing of the intrinsic deflections (cf. fig. 9). A difficulty arises sometimes, however, in differentiating complex types of impure flutter from fibrillation, especially when the rate lies within ranges possible for both conditions (cf. fig. 6). In both instances, the intrinsic deflections are irregular in incidence and contour. Judgment in classification of such types must necessarily be based on the rate of intrinsic deflections and whether the majority of deflections are similar in kind and form. Thus figure 6 is placed in the class of fibrillation on account of the high rate of the intrinsic deflection (600 per minute). Any error that may arise in this connection is not of fundamental significance, however, in the interpretation of contraction phenomena, for in both instances the excitation wave passes through the tissue investigated, in sinuous courses.

The interpretation of myogram tracings. A study of the actual contraction processes in flutter and fibrillation requires that we separate those shortening and lengthening phenomena due to actual muscular activity from those which are passively created by ventricular activity. As was previously emphasized in a paper with Niles (3), by far the greater number of variations in the length of auricular tissue during fibrillation are produced passively and not by active contraction processes. For this reason we must clearly recognize such passive effects. This can be

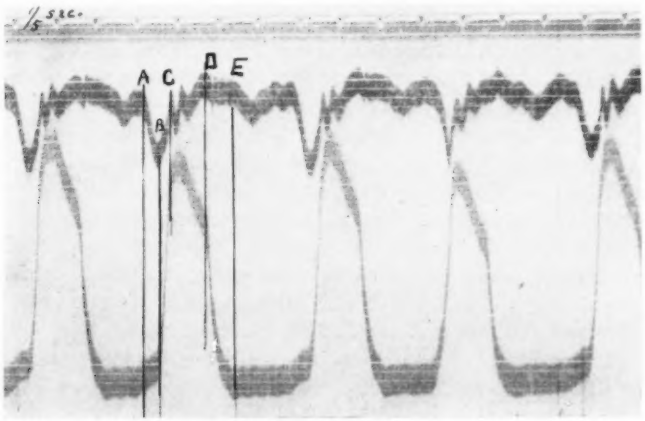


Fig. 1. Synchronous records of right auricular myograms (shortening = downstroke) and left intraventricular pressures to show effect of ventricular activity on length of auricular muscle. *A-B* active shortening of auricular systole, *C*, onset of ventricular ejection causing first lengthening then shortening of auricle; *D-E*, isometric relaxation of ventricle, *E*, beginning of inflow phase causing passive shortening of auricular muscle. In the fourth beat a premature systole shows the pronounced lengthening effect of ventricular ejection on the auricle.

done to a certain degree by restudying normal auricular myograms, such as shown in figure 1 for example. In general, if the ventricle exerts any traction, it tends first to produce a quick stretching of auricular muscle at the time of the ventricular ejection (*C*). This causes a sharp upstroke in the curve (i.e., lengthening of auricular tissue) which is frequently followed by a recoil so that a definite wave effect is produced during systole. During the isometric relaxation phase of the ventricle, *D-E*, the auricle may again be stretched or it may not, but, as the blood flows from auricle to ventricle during the

rapid inflow phase of early diastole, *E*, a passive decrease in the auricular length always occurs. Two things are apparent: *1*, when we have vigorous ventricular action it is unsafe, without other good evidence, to interpret as auricular contraction phenomena any waves that occur either during ventricular systole or at the time of ventricular filling early in diastole; and 2, that in consequence of this, records in which ventricular beats occur rapidly can usually not be employed. This, of course, makes it necessary in an analysis of this kind to discard totally a large proportion of records. In others only certain waves can be considered with safety.

After carefully scrutinizing a large number of myograms taken during auricular flutter and fibrillation (during which considerable experience has been acquired in diagnosing waves) I have come to the conclusion, however, that not all records in which the ventricular beat is rapid need to be entirely excluded. In these cases, many ineffective systoles occur which have apparently no effect on the length of auricular tissue. Much also depends on the vigor of auricular contraction in such cases. If these contractions are fairly powerful they may overpower the passive effects produced by ventricular changes during filling. In such cases, waves result which still give a correct indication of both the beginning and end of a contraction process although their contour is usually modified. The manner in which such interpretations are made will be illustrated as we go along. Always, however, our final conclusions must be based on waves that with reasonable certainty may be taken to indicate "active contraction waves" and not on these that may even be suspected of being expressions of or influenced by "passive variations."

THE NATURE OF AURICULAR CONTRACTIONS IN FLUTTER AND FIBRILLATION. *The auricular contractions in flutter.—When flutter is pure or only slightly impure, each centrifugal excitation wave is followed, after a fairly constant latent period, by a true muscular contraction. These contractions differ from normal in their greater variability in gradient and smaller amplitude, while their duration is distinctly shorter.* The following experiments supply examples of tracings upon which these conclusions are based and also illustrate the methods employed in evaluating the records:

Unfortunately, my collection contains no case of pure or even slightly impure flutter in which the ventricular rate is slow, therefore the number of unquestionable "active contraction waves" in any record is necessarily small. The illustration in figure 2 in which the flutter rate

was about 420 is selected for first analysis because the ventricular responses in this section of the curve recur somewhat irregularly and by that fact create a number of longer diastolic intervals. In this case the myogram records as well as the intrinsic electrical deflections were recorded from points on the anterior surface of the right auricle 16 mm. apart. The arrangement of the recording apparatus in this and all other illustrations was such that a shortening of auricular muscle causes a downward stroke on the curve.

In analyzing such records, it is necessary first to eliminate all waves occurring during the interval between systolic ejection and ventricular filling. By the aid of sound waves in the upper tracing or vibrations

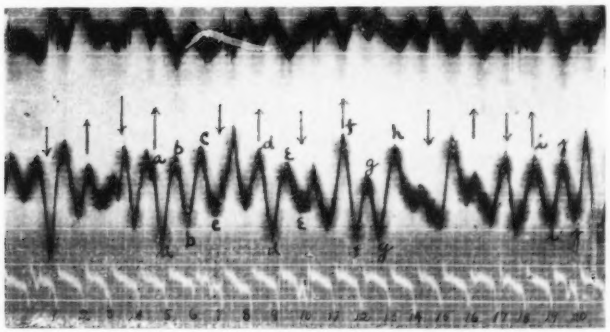


Fig. 2. Auricular myograms (middle) and intrinsic deflections (lower) in slightly impure flutter. Arrows directed downward, beginning of ventricular systole, arrows directed upward, approximate beginning of diastole. Numerals and letters referred to in text correspond to those in plot of figure 3. Waves *b*, *c*, *g*, *h*, *i* and *j* give an idea of waves due to contraction alone. Time, 0.05 second.

and sharp deflections in the myograms themselves, the beginning and end of each systole can be approximately established, and have been indicated by two sets of arrows respectively. When the waves between the arrows are eliminated it leaves us the diastolic myogram downstrokes *a*-*b*-*c*-*d*-*e*-*f*-*g*-*h*-*i* and *j* for consideration. Of these, however, the descending curve of *a*-*d*-*f* and possibly *i* are influenced if not entirely due to rapid ventricular inflow. Further, wave *e*, while reliable as to its onset is undoubtedly terminated prematurely by the effect of a succeeding systole. We shall refer to these as the "suspicious group" of diastolic waves. The time relations between all the mechanical waves recorded and the intrinsic electrical deflections are studied next.

To do this, a plot, such as is shown in figure 3, is made in the following way: The consecutive intrinsic deflections are first marked by an arrow and numbered 1-2-3-4-5, etc., to correspond with the deflections in the original curve. Between these are written the values for the inter-intrinsic intervals, *I. I.*[1] The beginning and the end of each contraction is next plotted in the case of waves *a* to *j* and related to the intrinsic deflections. These points are connected by a diagonal line which gives the duration of the shortening process. The waves (*b*, *c*, *g*, *h*, *i*) accepted as true contraction phenomena, are differentiated by a heavier solid line. Finally, the onset of each auricular shortening falling during the periods of ventricular systole is also plotted and indicated by broken lines. The actual numerical values thus determined for the latent period and duration of shortening are written directly on the plots. The following facts are established in this way:

1. The inter-intrinsic deflection intervals recur regularly (0.152 to 0.16 second), indicating that fundamentally the flutter is pure.

2. The latent periods of the pure contraction waves range from 0.04 to 0.05 (one doubtful value of 0.058), indicating that if the mechanical contractions could all be recorded as pure waves their sequence would be almost as regular as the intrinsic electrical deflections. This is further substantiated by the fact that the latent period of the suspicious diastolic waves (light lines in chart) also range from 0.044 to 0.05 second.

3. The duration of the pure contractions which range from 0.064 to 0.076 second indicates considerable uniformity in the contraction processes. These waves differ from normal in their smoother gradient and in the shorter phase of contraction (normal 0.09 to 0.096). Of the suspicious diastolic waves three show contraction phases within this range, the two others are distinctly longer. This shows that occasionally the incidence of ventricular filling and auricular emptying may act to deform not only the contour but also the duration of the shortening process.

4. The auricular waves occurring during ventricular systole follow excitation waves after latent intervals ranging from 0.032 to 0.076 second although many have values with the ranges of pure contraction waves above indicated. When all the waves of a myogram in pure flutter are plotted we must expect variations of about 0.03 second in

[1] The galvanometer connections were so arranged that negativity over the proximal arm of the myocardiograph was signaled by a negative deflection and its appearance over the distal electrode by a positive wave, i.e., in Lewis' terminology, the intrinsic deflections consist of downstrokes.

the spacing of the myogram waves due to passive ventricular effects and the interpretation of the condition as that of pure flutter can only be made on the regularity of a number of recurring contractions during long diastoles.

When the ventricle follows the auricle in 2:1 rhythm at a rapid pace no pure contraction waves may occur. The interference between changes in length due to pure contraction processes and passive effects may indeed become so great that it is quite impossible to establish a diagnosis on the basis of myograms alone. Such an instance is given in segment B, figure V, of a previous communication with Niles (3). In this case, not only are the contours of the auricular waves modified by passive effects but the rhythm, as indicated by the resultant curves, is also irregular.

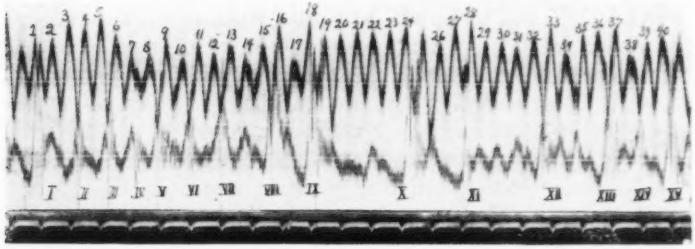


Fig. 4. Auricular myogram (upper) and heart sounds from interior of right auricle showing nature of auricular contractions during flutter when ventricular action is rapid. Numerals correspond to figure 5 and are referred to in text. *20–24*, *29–31*, = pressure waves. Time, 0.02 second.

On the other hand, the passive effects may interfere so fortunately that neither the rhythm nor the duration of the true contraction is appreciably affected. Such a case is shown in figure 4. In this experiment, the flutter rate was about 560 per minute. In the curve are shown with great clearness at least two groups of pure contraction waves, viz., waves 20 to 24 and waves 29 to 32. Inspection shows at once that, while the waves of each group are quite similar in form and amplitude, there are slight differences in the gradients of individual beats, as well as marked differences in the amplitude of the two wave groups. As compared with normal contractions, their amplitude varies from 62 to 70 per cent of the nor nal waves previously recorded, indicating, of course, that the auricular contractions are fairly vigorous in flutter. When measured and plotted, as in figure 5, these pure con-

traction waves (heavy lines) recur with remarkable regularity, the inter-contraction intervals ranging from 0.096 to 0.106 second and the duration only varying from 0.05 to 0.058 second.

Aside from these waves and with the possible exception also of waves 15, 17, 35, 36 and 40 (which may be regarded as pure in their earlier portions, and therefore reliable in regard to the onset of contraction only), there are no other waves in the entire record which are not subject to possible passive effects occasioned by ventricular ejection or filling. The contour of these waves naturally does not indicate the nature of the contraction processes. It is interesting to note, however, that when all the summits and valleys of the consecutive waves are plotted, as shown in figure 5, there is still a marked degree of regularity. The majority of the inter-contraction intervals range between the values given above for the pure beats. In fact, there are only 5 instances in which the values are more than 0.006 of a second above or below these figures. The durations of the waves affected by ventricular contractions are also surprisingly constant and in a large proportion of cases equal those found in pure beats. The greatest variations range from 0.04 to 0.06 of a second as compared with 0.05 to 0.058 second in pure waves. From this we may conclude that, while the ventricular contractions modify the contour of auricular waves greatly, there are cases of auricular flutter in which the individual auricular contractions are sufficiently vigorous to make their beginning and end distinctly legible in the myogram tracings.

The contraction process in impure flutter and fibrillation.—As we pass through the transition stages existing between pure flutter and fibrillation of the clinical type and as the irregularity in the spread of the excitation waves progressively increases, it is found that coördinated auricular contractions still occur but that, in general, they become smaller as the type of disorder advances. They differ from the purer types of flutter, first, in that the latent periods as well as durations and amplitude vary more from beat to beat and finally, in cases frankly corresponding to clinical fibrillation, contractions may occasionally be omitted. As the rate of excitation increases further and the condition borders on a state of rapid reëxcitation, all efforts at coördinate contractions disappear and at this stage alone can the auricle be said to contract in a fibrillary manner. These conclusions are illustrated by an analysis of the following experiments:

The detailed relations of myograms to excitation waves derived from the points to which the myocardiograph arms were attached, are illustrated in figure 6. In this case the intrinsic deflections varied in dif-

ferent sections from 600 to 720 per minute while the ventricular rate was about 60. This gives many pure contraction waves for study purposes.

The irregular spacing and contour of the intrinsic deflections is evident upon inspection of the original record but is even better illustrated in the plot shown in figure 7. The inter-intrinsic intervals vary from 0.070 to 0.120 second.

If the various pure contraction waves occurring during ventricular diastole are plotted in relation to these excitations, it is obvious that small mechanical responses follow the majority of excitations after latent periods which range from 0.02 to 0.06 second. Many excitation waves, however, are followed by no mechanical shortening whatsoever (e.g., *a-h-j-r-t-v-x*, etc.). It is obvious, therefore, that, in contrast to

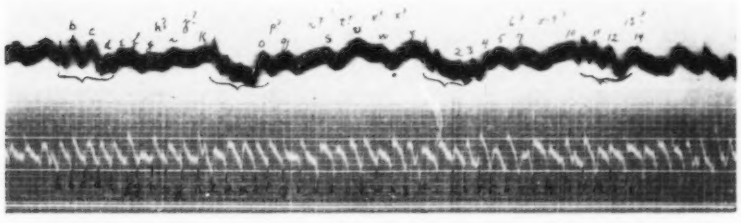


Fig. 6. Myogram (upper) and intrinsic deflection (lower) in auricular fibrillation accompanied by A-V block and slow ventricular action; letters and numerals correspond to figure 7 and are referred to in text. Time, 0.05 second.

flutter, neither the rate nor the rhythm of excitation is indicated by the inter-contraction intervals.

The duration of the pure contractions vary extremely (0.03 to 0.125 second) but average from 0.045 to 0.055 second—periods not greatly different from those observed in flutter. Inspection of both the plot and the records shows that a few excitation waves which fail to evoke a contraction fall at a time when the auricle is already contracting. This is clearly the case in waves *h-j-v*, 6 and 8. It may be suggested that since these particular auricular contractions are prolonged (e.g., 0.09 to 0.110 second) the muscle is actually responding to both excitations by a sort of smooth summation. Such is not necessarily the explanation of the failing contraction for the mechanical responses also fail to occur after previous contractions of short duration (e.g., 1, *r*).

When the auricular tissue is excited at rates above 720 (approximately), the mechanical responses fail to a greater degree. Such an

instance is shown in the records of figure 8. The auricle from which this tracing was taken appeared dilated and gave only fine fibrillary twitchings. The curve shows intrinsic deflections of an extremely irregular order which recur about 900 times per minute. The condition may therefore be said to border on that of rapid excitation, but, owing to the high degree of irregularity, could also be classified as a state of fine fibrillation. The myogram record and intrinsic deflections show no correspondence, the only mechanical movements being gradual variations occurring at longer intervals of time. The large deflections *a-b* are clearly due to passive effects of ventricular ejection and filling.

A study of the pure contraction waves occurring during diastole in this large series of experiments, of which the published figures are but samples, leads to the conclusion that, as the rate of excitation increases

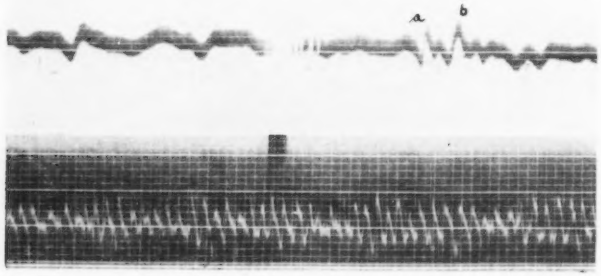


Fig. 8. Myogram (upper) and intrinsic deflections (lower) in rapid reëxcitation. Time, 0.05 second.

and as the intrinsic deflections become more irregular in spacing and contour, the coördinated contractions first become smaller, then occasionally lapse and finally at high rates disappear entirely or are indicated at most by very slight changes in length which bear no relation to the excitation waves.

It happens many times, however, that both inspection and graphic records show that much coarser waves occur in the auricle during conditions undoubtedly corresponding to clinical fibrillation. Such a curve is reproduced in figure 9. This happens only when the ventricular rate becomes high. Is it possible then to have cases of clinical fibrillation in which coördinated contractions occur which are fully as large and vigorous as those maintained in flutter? This question has been previously analyzed by Niles and myself. We concluded from in-

spection and actual records of ventricular tugs that such waves do not represent true auricular contractions but are largely the passive effects of ventricular action. It appears desirable, however, to test this conclusion again, from another angle. If any or all of these waves truly indicate contraction phenomena of the auricle they should qualify in regard to three criteria: *1*, they should be related to excitation waves following them at intervals not much more variable than those indicated in the previous; *2*, they should qualify as to form, viz., not show sharp angles and gradients; *3*, they must not coincide with the periods extending from ventricular ejection to ventricular filling. In order to study curves according to these criteria, all the summits and valleys on the myogram shown in figure 9 were plotted both as regards the incidence of ventricular ejection and filling and also in relation to the

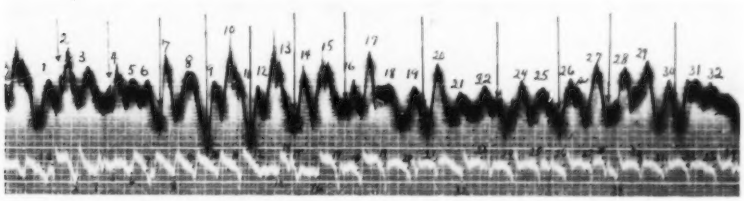


Fig. 9. Myogram (upper) and intrinsic deflections (lower) in auricular fibrillation with rapid and vigorous ventricular action. All waves with exception of nos. *1*, *5*, *19* and *22* affected by passive ventricular influences. Numerals correspond to those in figure 10 and referred to in text.

incidence of the intrinsic deflection whether up or down. The frank discrepancy between intrinsic deflections and auricular changes is obvious at a glance. In the chart shown as figure 10 these mechanical changes are related to the most consistent electrical variations. From this, table 1 was constructed and *failure of acceptance* on the basis of each criterion above mentioned is indicated by a cross. A glance through this table shows that 12 waves are disqualified according to all criteria, 14 according to two criteria. This leaves 6 waves, of which 2 occur during systole (no. 5, no. 26) and 4 during diastole (nos. 1, 19, 22, 30) which may be regarded as pure at the time of onset. Of these the downstroke of 19 and 30 are undoubtedly distorted by the next ensuing ventricular systole so that their amplitudes probably also are not trustworthy. This leaves us only waves 1, 5 and 22 as giving clear ideas of the extent of the auricular contraction—which is that only very

TABLE I

Showing evaluation of waves in fibrillation curves

NUMBER OF MYOGRAM WAVE	APPARENT LATENT PERIOD	TIME OF OCCURRENCE	CONTOUR
1	0.036	Diast.	Accept
2	0.10 X	Sys. X	X
3	0.07	Inflow X	X
4	0.132 X	Sys. X	X
5	0.05	Sys. X	Accept
6	0.006 X	Inflow X	X
7	0.064	Sys. X	X
8	0.08 X	Inflow X	X
9	0.005 X	Sys. X	X
10	? X	Sys. X	X
11	0.022 X	Inflow X	X
12	0.006 X	Sys. X	X
13	0.01 X	Sys. & Inflow X	X
14	0.046	Sys. X	X
15	0.064	Inflow X	X
16	0.11 X	Sys. X	X
17	0.064	Sys. X	X
18	0.048	Inflow X	X
19	0.04	Diast.	Accept
20	0.016 X	Sys. X	X
21	neg. X	Inflow X	Accept
22	0.024 X	Diast.	Accept
23	?	Sys. X	X
24	0.048	Sys. X	X
25	0.036	Inflow X	X
26	0.052	Sys. X	Accept
26a	0.022 X	Sys. X	X
27	neg. X	Inflow X	Accept
28	0.062	Sys. X	X
29	0.060	Inflow X	X
30	0.046	Diast.	Accept
31	0.042	Sys. X	Accept

small coördinated contractions do exist in this type of fibrillation. These particular contractions had the following time relations:

WAVE NUMBER	LATENT PERIOD	DURATION
1	0.036	0.044
5	0.05	0.054
19	0.04	0.05
22	0.024	0.042
30	0.06	0.056

We may conclude, therefore, that at rates of excitation occurring in conditions comparable to clinical fibrillation, the contractions may still be coördinated but are always feeble. The large waves occurring when ventricular rate is rapid are entirely due to passive effects which entirely obscure the small true contractions which exist.

The relation of ventricular systole to auricular contraction. In the normal cardiac mechanisms, ventricular systole signaled by an increase in ventricular pressure or by synchronously recorded heart sounds occurs either at the moment that contraction of the auricle reaches its end or shortly thereafter (0 to 0.063 second, average 0.021 (5)). Never has it been found in my records that it precedes the end of auricular shortening, as held by Lewis, Feil and Stroud (2).

When in pure flutter, the ventricle responds regularly in 2:1 or 3:1 rhythm, the relation of auricular and ventricular systole remains quite regular. It differs from the normal, however, in that ventricular systole often slightly precedes the termination of auricular contractions. Such relations are shown in ventricular beats *II*, *III*, *V*, *VI*, *VII* and *VIII* of figure 4 and the corresponding plot of figure 5. This relation may be interpreted either as due to the fact that the central ring which gives rise to the impulses lies nearer to the ventricle or that the more distant tissues contract later.

When ventricular block is irregular and usually also when the degree of block is greater, this exact relation does not obtain even when the flutter is otherwise pure. Such waves are shown in beats *IX*, *X*, *XI*, *XII*, *XIII* and *XIV* of figures 4 and 5 where ventricular systole is seen to fall before and during as well as toward the end of auricular systole. It is of course quite impossible to say whether this is due to irregular transmission through the auricular tissue or through the His-Tawara and Purkinje systems—but whichever explanation is adopted, it is apparent that the first evidences of conduction disturbance appear in this irregular relation of auricular and ventricular beats. Practically, this actual variation in the A_s-V_s intervals is of importance in partly explaining perhaps that lack of precise spacing so frequently found in arterial pulse tracings derived from clinical cases of pure flutter.

During advanced states of impure flutter and during conditions corresponding to clinical fibrillation, all semblance of relation between auricular and ventricular beats is usually lost. This is well illustrated in the graphic charts of figures 7 and 10 and is of course precisely what might be expected when the excitation waves are transmitted over different portions of auricular tissue in sinuous courses of varying complexity.

THE SIGNIFICANCE OF VARIATIONS IN AMPLITUDE, DURATION AND GRADIENT OF THE TRUE AURICULAR CONTRACTIONS RECORDED IN FLUTTER AND FIBRILLATION (Theoretical). We have seen that what may be regarded as coördinate auricular contractions occur more or less regularly in all types of auricular irregularity ranging from flutter to fibrillation corresponding to the clinical type and that only at excitation rates bordering on the state of rapid excitation do such attempts at coördinated beats cease entirely.

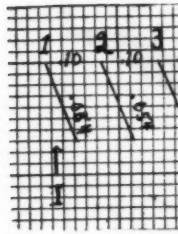
The recurrence of contractions of such regularity cannot be explained satisfactorily on the basis that clinical fibrillation results from stimuli sent out from multiple foci and, in general, supports the view that they are due to an underlying excitation by centrifugal waves derived from a central circus movement. We must examine further, however, how the details of the contraction phenomena can be interpreted according to this mechanism of excitation.

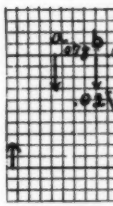
How are the shorter durations of the pure auricular contractions in flutter and fibrillation to be explained? Are they capable of interpretation by supposing that an earlier balance of contracting and relaxing units takes place, or must we assume that the duration of each fractionate contraction is also shorter? Is the progressive decrease in amplitude of auricular contraction as we pass from flutter to fibrillation to be attributed to irregularity in excitation and response of the individual fractions or must we suppose that the fundamental functions of contractility or irritability are also depressed?

Since we have no records in which contractions even approximating fractionate contractions were recorded, our answer to these questions must remain probable rather than quite certain. Nevertheless, a logical analysis of the contraction phenomena above present is suggestive as to the nature of the changes which are concerned in the contraction phenomena recorded during flutter and fibrillation.

Let us picture, as in figure 12, a series of diagrams indicating the nature of the excitation phenomena under different conditions of auricular irregularity. Let lines X and Y represent points (16 to 20 mm. apart) which approximate during any contraction. Let us further suppose that between these lie inter-communicating lines of auricular fractions and that the arrows indicate roughly the paths of impulses through this tissue. We may now consider the contraction phenomena under different conditions presented in this diagram.

The normal contraction (fig. 12, *A*). Normally, impulses pass straight through the theoretical columns of muscle fractions from X to Y at





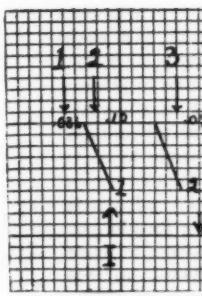


Fig. 3. Plot sho
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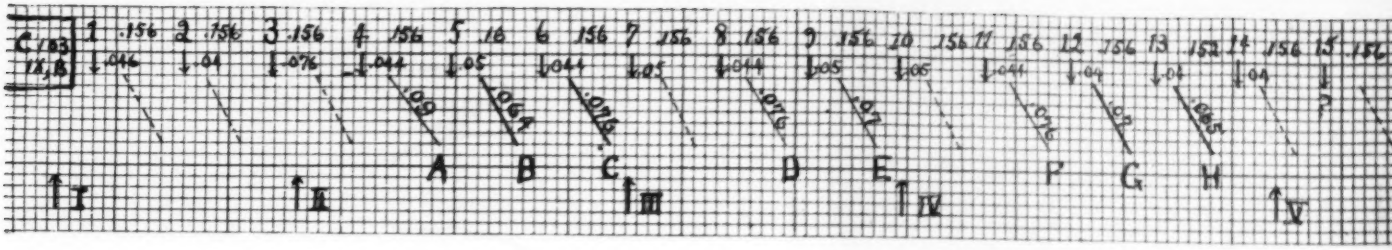


FIG 3

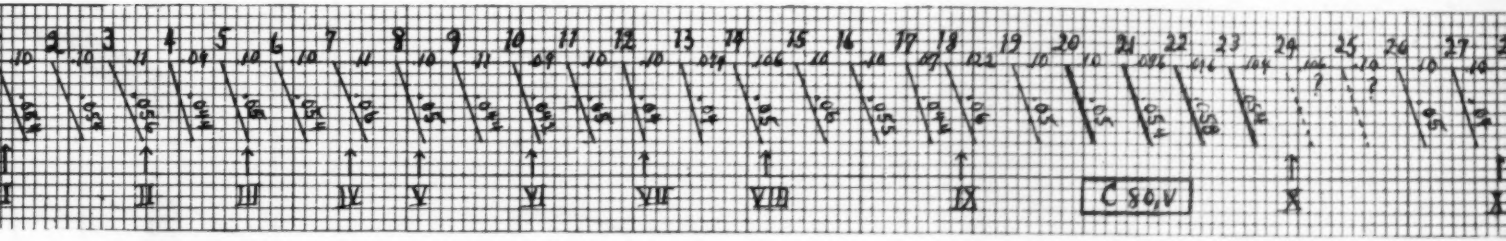


FIG 5

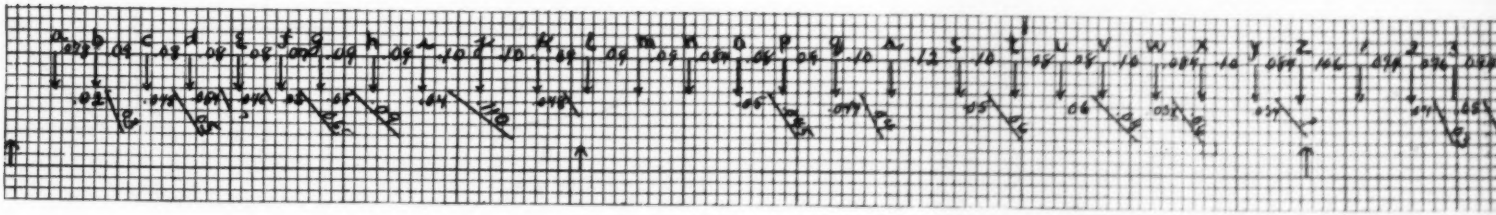


FIG 7

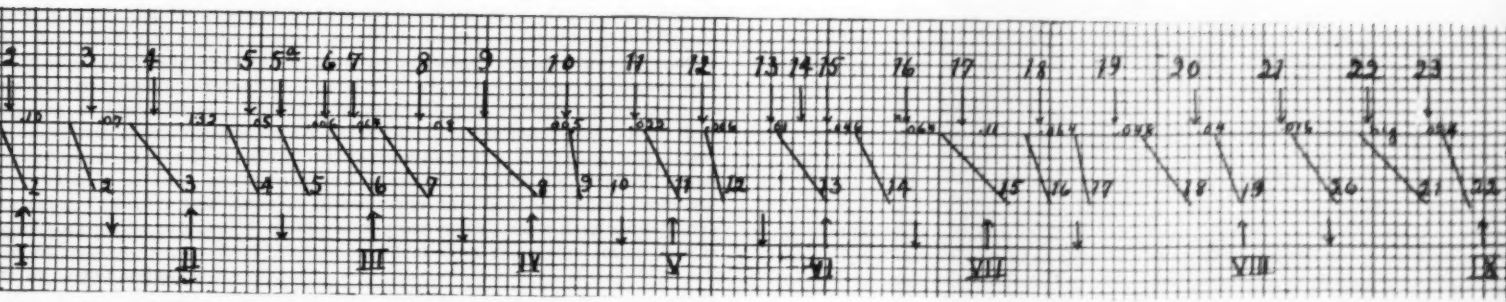
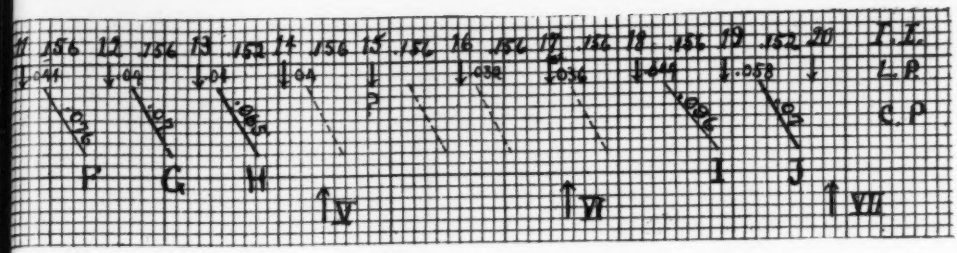
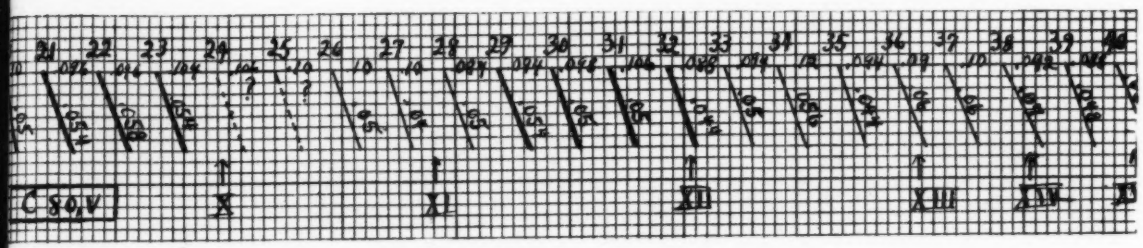
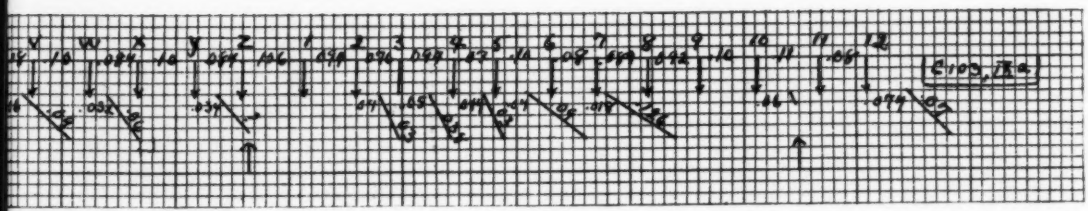


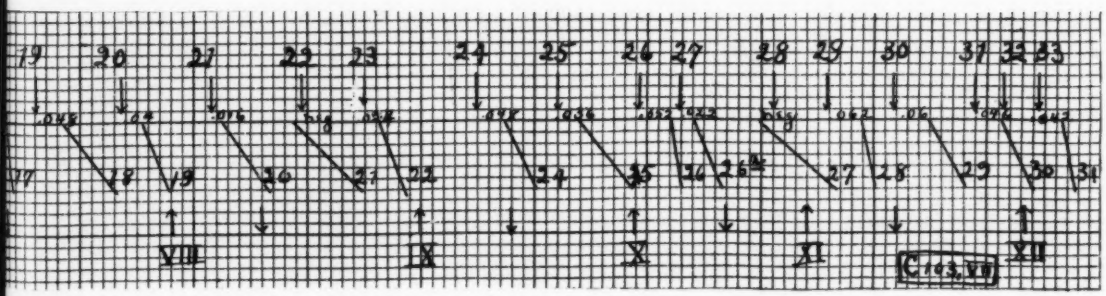
FIG 10

. 3. Plot shows a regularity of inter-intrinsic intervals *I–I*; latent period, *L.P.*, and contraction phase, *C.P.*, of beats labeled to correspond in figure 2. Arrows a
. 5. Plot constructed and labeled as in figure 3. Numerals and data relate to those in figure 4.
. 7. Plot constructed and labeled as in figure 3. Numerals and data relate to those of figure 6.
. 10. Plot constructed and labeled as in figure 3. Numerals and data relate to those of figure 9.









ts labeled to correspond in figure 2. Arrows and Roman numerals *I*, *II*, *III*, etc., onset of ventricular systoles.

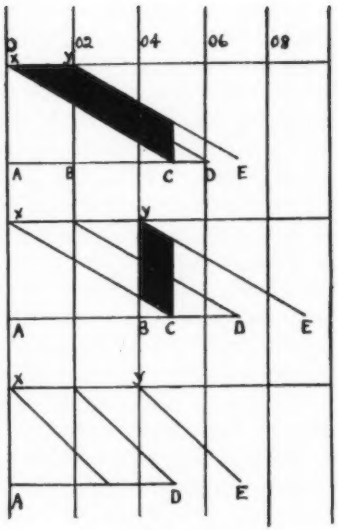


Fig. 11

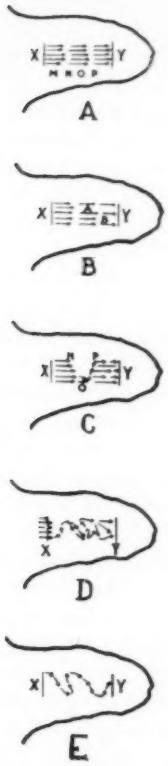


Fig. 12

Fig. 11. Diagram showing how rate of excitation, and duration of fractionate contraction influences amplitude, gradient and duration of total contraction in auricle. *X–Y*, transmission of excitation process in imaginary strip (= 0.02 second in upper and 0.04 second in middle and lower records). Oblique horizontal lines, *X–C*, *Y–E* etc., represent duration of fractionate contractions (= 0.05 second in upper and middle records; 0.03 second in lower). Shaded area represents overlap of fractionate contraction, duration of which determines amplitude and steepness of contraction. Observe how shortening of fractionate contraction combined with slower conduction abolishes all overlap in lower plot. *B–C*, duration of phase in which concerted contraction obtains. *D* in each case determines balance between contracting and relaxing fractions, i.e., end of actual shortening. Observe that decrease in excitation rate in itself causes prolongation of contraction process (cf. middle and upper curve), while decrease in duration of fraction contractions has reverse effect even when conduction rate is slow (cf. lower and upper curve).

Fig. 12. Five diagrams illustrating the possible spread of the excitation waves through a strip of tissue *X–Y*; *A*, normally; *B*, during pure flutter; *C*, during impure flutter; *D*, during fibrillation of the clinical type; *E*, during advanced grades of fibrillation (rapid excitation); areas through which arrows do not run supposed to remain unexcited. (Description in text.)

a velocity of 800 to 1000 mm. per second (Lewis). We may suppose that as soon as all fractions between X and m have started to contract, sufficient force has accumulated to start the shortening of the whole strip X to Y. As the fractionate contractions start at n, o and p and finally at Y, these additions cause the curve to increase in steepness and to maintain its steepest gradient as long as all fractions continue in this contractile state. It is obvious, then, that as long as all fractions are excited, the steepness of the gradient is determined by the rapidity with which the contraction wave spreads, viz., the rate at which individual fractions are summated (cf. also fig. 11). As soon as the fractions near X have finished their contractions, a relaxation wave sweeps over the muscle fractions from X to Y. As this wave of relaxation reaches n, the gradient of contraction becomes slower and as it arrives at o, we may suppose that a balance of contracting and relaxing fibers determines the end of the shortening process in the strip X and Y.

The contraction process in pure flutter (figs. 11 and 12, B). In this condition, it is necessary to explain why contractions of smaller amplitude and shorter duration occur, when impulses spread regularly but at reduced rates through an auricular strip. While it is not possible to interpret these changes with any degree of positiveness, certain tentative conclusions may be arrived at by a process of exclusion.

In the first place, the possibility may be considered that these changes are the result of an earlier balancing of contracting and relaxing fractions, brought about by a fundamental depression of conductivity. Let us suppose that the impulses are conducted from X to Y as normally but at a slower rate (fig. 12, A). The effect is illustrated in the upper and middle curves of figure 11 where it is assumed that the duration of each fractionate contraction remains of normal length. It will be noticed that the time during which all fractions of muscle are acting together (shaded block) is very much abridged as is also the rate at which new contractile elements are added to the old contraction process. This has the effect of producing a more gradual gradient and through this, a reduction in the total amplitude of contraction. This accords with our findings. It will also be noticed, however, that such an effect causes a definite lengthening of the time when a balance between contracting and relaxing elements takes place, in other words if this were the only effect produced during flutter *we should expect that the duration of the entire contraction process would be somewhat lengthened rather than, as is actually the case, appreciably reduced.* Furthermore, Lewis, Drury and Bulger (4) have submitted experiments which indicate that the rate of impulse

conduction remains unaltered and that the delay in transmission is due entirely to the small barrier of refractory muscles which obstruct or delay the passage of the impulse.

In the second place, it may be supposed that certain fractions remain refractory to excitation and therefore do not enter into the contraction process. Thus, if we suppose, as in figure 12, B, that the excitation waves pass through the strip *X*-*Y* on certain routes but not over the areas labeled *A* and *B*, we could account for the similar intrinsic deflections as well as the small amplitudes of the contractions. Were this the case, however, we should expect to find evidences of alternation in the contractions and the intrinsic deflections should show greater variation in amplitude from beat to beat.

Finally, it is conceivable that all fractionate contractions enter into the reduction of amplitude but that each fractionate contraction is not only less effective but of shorter duration (fig. 11, lower illustration).

The contraction process in impure flutter (figs. 11 and 12, C). In addition to the phenomena analyzed above, it is necessary to further inquire why the amplitude and contour of the auricular contraction vary so greatly in consecutive beats. Such variations can readily be assigned to irregularity in the summation of fractionate contractions when the rate of impulse transmission is irregular. As the rate of auricular responses increases and the cycle shortens, the absolute refractory phase does not shorten proportionally and the remainder may even become partially refractory. Consequently, the muscle fractions are not all excitable and thus block the impulses, which are then forced to seek roundabout routes through excitable tissue. Thus, let us suppose, as shown in figure 12, C, that a series of blocks develops along all the muscle fractions along the front *n* - *o* with the exception of a small bridge of tissue indicated at *o*. Obviously, the time required for the excitation wave to travel over the paths from *n* to *p* is much greater than when conduction is straight. It is obvious also, however, that by virtue of some such sinuous path occurring at one or several places, the fractions in the wedge-shaped arc (*n o p*) are not excited to contract at the proper time and therefore do not participate in the shortening of the segment *X*-*Y*. The total amplitude of this contraction will therefore be smaller by the number of fractions thus thrown out of action and will be irregular in gradient whenever and wherever such an elimination occurs. Furthermore, owing to the delayed excitation of the contraction front *o* - *p*, the fractionate contraction toward the *X* side of the gap will have progressed further or even be terminated when

those toward the *Y* side begin. This will hasten the moment when a balance between contracting units occurs, *i.e.*, shorten the duration of total contraction in any strip of muscle such as reported by *X*-*Y*.

It is of course quite impossible to express with entire accuracy the details of the mechanisms in diagrams. When such blocks occur at irregular times during the spread of the excitation wave and in varying localities from beat to beat as in impure flutter, it is obvious that no effect other than great variability in the amplitude and contours of the contractions can occur from beat to beat.

The contraction process in fibrillation. All the factors mentioned as operating to reduce the amplitude of coördinated contractions and make them irregular, act in a greater degree to produce the small and irregular contractions in clinical fibrillation. Thus, if we suppose that the excitation wave follows the irregular course shown in figure 12, D, it is obvious that not only do many fractions remain unexcited, but that those excited begin and end their contractions in such an unordered manner that they tend to counteract rather than summate each other. Both the absence of mechanical response and great variations in duration may also be readily explained by the great variability in the excitation paths from beat to beat. Thus, if we recall that the excitation wave may turn upon itself or that other waves may enter at points other than *Y*, it can readily be understood why occasional contractions of unusual length but small amplitude occur, or why they occasionally neutralize each other and produce no shortening whatsoever.

From such cases, we pass to those found on the borderline of rapid excitation, when the amount of tissue that remains responsive to the advancing wave is so small that the muscle strip virtually becomes a conducting mechanism and ceases to be a contracting strip in the sense that it is able to produce even minute shortenings of the auricular muscle (cf. fig. 12, E).

SUMMARY

1. By careful relation of the optical myogram curves recorded from points 16 to 20 mm. apart on the auricle to ventricular ejection and filling on the one hand and to the intrinsic electrical deflection recorded from the same points, it is possible to separate the waves due to "active contraction phenomena" from those passively caused by or influenced by ventricular activity.

2. In pure or slightly impure flutter, each excitation wave is followed after a fairly constant latent period (0.04 to 0.05 second) by a coördi-

nated muscular contraction differing from normal in its slower gradient, smaller amplitude, shorter duration and a certain variability in amplitude. In pure flutter, groups of waves of different size obtain from time to time but successive beats are fairly regular, in simpler forms of impure flutter, the contractions vary in amplitude from beat to beat.

3. Theoretical considerations indicate that it is possible to explain some of these phenomena only by assuming that the amplitude and duration of the fractionate contractions are reduced. It is probable, however, and in accord with the interpretation given to the delayed transmission by Lewis and his associates, that the changes in duration and amplitude are chiefly due *a*, to an entire elimination of certain contractions; and *b*, to the neutralization of others by simultaneous relaxations.

4. In flutter of great impurity as well as during conditions corresponding to clinical fibrillation, the coördinated contractions which still follow the majority of excitation waves become smaller in amplitude and more irregular in size and duration. A relatively small number of excitation waves fails to elicit a mechanical response. Theoretical considerations indicate that the smaller amplitude as well as irregularity in size and duration may be accounted for by the more or less irregular elimination of certain muscle fractions from the contraction process. The total amplitude of any wave will therefore be determined by the number of fractions that fail to be excited and the gradient will be irregular whenever and wherever such an elimination occurs.

5. In high degrees of fibrillation, bordering on states of rapid excitation, the auricle no longer responds to separate excitation waves. The contraction phenomena recorded consist of very small and often barely recognizable waves lasting for intervals occupied by numbers of excitation waves. Under these conditions, we may suppose that the fractions excited at one time are so small in number that the auricle practically becomes a conducting rather than a contracting structure.

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STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION[1]

VI. The Effect of Unilateral Section of the Splanchnic Nerve on the Elimination of Certain Substances by the Kidney

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Received for publication July 18, 1922

In work reported in previous papers of this series, it was shown that the effect of section of the splanchnic nerve on the elimination of various substances by the kidney could be explained by the increased blood flow occasioned by the loss of vasomotor control of the blood vessels (1). This has since been confirmed by Yoshimura (2), using the same method which Marshall and Kolls employed. It was then concluded that section of one splanchnic nerve gave a method for increasing the blood flow through one kidney, and studying the effects of this increase, using the other kidney as a control. Increasing the blood flow by this means caused a marked increase in the elimination of water and chlorides, a definite but smaller increase in that of urea, and practically no change in the output of creatinine and phenolsulphonephthalein (3).

The present paper presents an extension of this work to the study of the effect of increased blood flow on sulphate, phosphate, carbonate, ammonia and hydrogen ion concentration in the urine.

Methods. The methods as regards species of animal, anesthesia, operative technique and collection of urine were the same as those employed previously, and have been described (3). Urea, creatinine and chlorides were estimated by the methods used by Marshall and Kolls. Ammonia was determined by aeration, sulphates by the benzidine method as described by Drummond (4), phosphates by the colorimetric method of Bell and Doisy (5), carbonates by Van Slyke's method (6),

[1] A preliminary account of these observations was presented before the American Physiological Society at the Chicago meeting, December 28, 1920. See Proceedings, this Journal 1921, lv, 278.

and hydrogen ion concentrations by a modification of the procedure of Henderson and Palmer (7). In the determination of carbonates in urine (total carbon dioxide) and hydrogen ion concentrations where dilute urine are encountered special precautions in the collection of the urine are necessary (8). These precautions were not sufficiently appreciated in many of the earlier experiments, so that these determinations, although carried out, are omitted from many of the tables, as we cannot guarantee their accuracy. Special experiments were preformed on the elimination of carbonates and hydrogen ions, where all necessary precautions were taken.

RESULTS: *Experiment 1.* October 22, 1920. Dog, male, weight, 10.0 kilos. At 10:50, left splanchnic sectioned just above the adrenal gland. Ureters cannulated. At 12:30, injected intravenously 25 cc. of 10 per cent sodium chloride solution.

TIME		URINE	NaCl	UREA	CREATININE	NH_4	PO_4	SO_4
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
11:30–12:30	R	4.5	26.8	254	8.27	15.8	38.2	24.0
	L	5.5	40.0	267	8.65	15.1	46.0	27.0
	$\frac{L}{R}$	1.22	1.49	1.05	1.05	0.96	1.20	1.17
12:30–1:30	R	13.0	144	196	6.80	10.7	84.4	
	L	21.2	275	235	7.12	11.4	99.6	
	$\frac{L}{R}$	1.68	1.91	1.20	1.05	1.06	1.18	

Experiment 2. October 28, 1920. Dog, female, weight, 9.1 kilos. At 10:00, left splanchnic was sectioned. Ureters cannulated. At 11:42, 20 cc. of 10 per cent sodium chloride solution injected intravenously.

TIME		URINE	NaCl	UREA	CREATININE	NH_4	PO_4	SO_4
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
10:40–11:41	R	2.0	14	37	4.00		20.1	14.0
	L	15.8	118	179	5.16		45.4	28.0
	$\frac{L}{R}$	7.90	8.43	4.85	1.29		2.25	2.00
11:41–12:41	R	11.0	125	139	4.76	3.52	26.7	12.4
	L	41.0	471	180	4.63	3.26	39.0	16.3
	$\frac{L}{R}$	3.71	3.80	1.29	0.97	0.93	1.46	1.31

Experiment 3. November 10, 1920. Dog, male, weight 11.0 kilos. At 12:45, left splanchnic sectioned. Ureters cannulated. At 1:22, given 20 cc. of 10 per cent sodium chloride solution intravenously.

TIME		URINE	NaCl	UREA	CREATININE	NH_4	PO_4	SO_4
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1:30–2:32	R	13.1	178	165	7.44	4.36	36.0	12.1
	L	24.0	328	239	8.00	4.36	45.5	15.5
	$\frac{L}{R}$	1.83	1.88	1.45	1.07	1.00	1.26	1.28
2:32–3:32	R	5.1	64	194	7.20	4.84	22.0	7.0
	L	23.2	285	262	7.04	5.45	33.5	10.5
	$\frac{L}{R}$	4.55	4.45	1.35	0.98	1.13	1.52	1.50

The above experiments are typical of many that have been carried out on the elimination of sulphates, phosphates and ammonia. They are selected because they are more complete than others for one reason or another, and present simultaneous data on chlorides, urea and creatinine. It is evident from the protocols and tables given below that ammonia resembles creatinine and phenolsulphonephthalein in its elimination, while phosphates and sulphates fall into the group with urea rather than in that with chlorides and water.

The two experiments given below are examples of those which were carried out on the elimination of carbonates and hydrogen ions. The carbonates are expressed as milligrams of carbon dioxide and include the free carbon dioxide present in the specimens of urine. The bound carbon dioxide (that present in the form of bicarbonates) has been calculated assuming that the bicarbonate is completely ionized, and using 4.4×10^{-7} for the dissociation constant of carbonic acid. The results are sufficiently accurate for the present purpose. The samples were all carefully collected under paraffin oil as described, and were analyzed within a few minutes after collection. Data on the behavior of phosphate and creatinine are presented in these experiments for comparison. It is obvious from the tables given that carbonates resemble chlorides and water, and that hydrogen ion concentration may be decreased or unaffected by increased blood flow. If the percentages of free carbon dioxide present in the specimens are calculated, they are found to be consistently lower on the operated side. The carbon dioxide tension in the urine has been taken as an index of the tension of this gas in the renal tissues. If this is so, it is obvious that the carbon

dioxide tensions in the kidney may vary with the same tension in the arterial blood. This disproves the hypothesis recently advanced by Marshall that the tension of carbon dioxide in the urine bears a simple relation to the tension of the gas in the aveolar air (8). Apparently increased blood flow through the kidney (section of the splanchnic nerve) lowers the carbon dioxide tension in the renal tissues.

Experiment 4. May 18, 1921. Dog, male, weight 17.0 kilos. At 10:15, left splanchnic sectioned. Ureters cannulated. At 11:10, given 40 cc. of 10 per cent sodium chloride solution. Urine collected under parrafin oil without coming into contact with air by means of long bent cannulae.

TIME		URINE	TOTAL CO_2	BOUND CO_2	pH	CREATININE	PO_4
		cc.	*mgm.*	*mgm.*		*mgm.*	*mgm.*
11:15–11:45	R	10.3	2.72	1.29	6.4	7.03	13.2
	L	21.4	4.36	2.07	6.4	7.00	22.2
	$\frac{L}{R}$	2.08		1.60		1.00	1.68
11:45–12:15	R	5.2	0.98	0.68	6.0		22.4
	L	17.2	1.72	1.19	6.0		34.6
	$\frac{L}{R}$	3.30		1.75			1.54

Experiment 5. May 27, 1921. Dog, male, weight 15.5 kilos. At 11:00, left splanchnic sectioned. Ureters cannulated. At 11:25, given 30 cc. of 10 per cent sodium chloride solution. Urine collected under parrafin oil.

TIME		URINE	TOTAL CO_2	BOUND CO_2	pH	CREATININE	PO_4
		cc.	*mgm.*	*mgm.*		*mgm.*	*mgm.*
11:30–12:00	R	11.5	2.32	0.95	6.2	9.2	24.6
	L	41.0	5.80	2.73	6.3	13.2	48.2
	$\frac{L}{R}$	3.56		2.87		1.43	1.96
12:00–12:30	R	21.3	3.03	1.12	6.1	16.8	
	L	48.0	6.10	3.20	6.4	17.1	
	$\frac{L}{R}$	2.26		2.86		1.02	

Discussion. The results reported in this paper offer a confirmation of the results reported on urea, creatinine and chlorides by Marshall and Kolls (3), and an extension of their work to other substances. The substances eliminated by the kidney can be divided into three groups—water,

chlorides and carbonates whose elimination is markedly increased by increased blood flow (section of the splanchnic nerve); creatinine, phenolsulphonephthalein and ammonia, which are unaffected in absolute amount by increased blood flow; and urea, sulphates and phosphates which are definitely increased but in less degree than the first group, thus occupying an intermediate position. In general, creatinine, phenolsulphonephthalein and ammonia are unchanged in absolute amount when the rate of urine flow from one kidney is increased by section of the splanchnic nerve; that is, the quantities eliminated by each kidney agree within 10 per cent, which is within the variation found in normal control animals eliminating about the same amounts of urine from each kidney. Occasionally, experiments are encountered in which the quantities of these substances eliminated are much greater from one kidney than the other. Since Marshall and Kolls (9) found the elimination of creatinine and phenolsulphonephthalein to depend on the amount of kidney tissue and not on the amount of blood flowing through the organ, provided this was above a certain minimum, these differences might be due to differences in the amount of functioning renal tissue in the two kidneys. Such might be suspected when the differences persist throughout the experiment, but where marked differences are encountered in one period and not in another one of the same experiment (as in periods 1 of experiments 2 and 5 of the present paper), another explanation must be sought. It was indicated in a previous communication that an extreme reduction in blood flow did reduce the amount of creatinine eliminated (9), and we have subsequently found that stimulation of the splanchnic nerve reduces the output of creatinine when the quantity of urine is markedly reduced. Moreover, partial asphyxia causes in an animal with one splanchnic nerve sectioned a marked decrease of urine on the normal side with a reduced output of creatinine, and a marked increase of urine on the operated side. This effect is produced by vasoconstrictor impulses passing to the kidney along the splanchnic nerve, and would probably be reflexly produced. Reflex stimulation and partial asphyxia, then probably account for these differences, probably through a marked diminution of the blood flow through the kidney of the unoperated side. It is possible that the reduced blood flow causes under these conditions closure of a certain number of glomeruli and hence renal units, as has been observed by Richards and Schmidt (10) in the frog's kidney. If the observations of these investigators can be transferred to the mammalian kidney, the intermittence of glomerular flow may account for the fact that the

elimination of the various constituents of the urine by the two normal kidneys of an animal is not at all the same if very short periods of collection are used, but is very nearly the same if longer intervals (one-half hour or more) are allowed for collection of urine.

Ellinger (11), in a recent article on the influence of nerve section on the elimination of substances by the kidney, finds after section of the splanchnic or renal nerves on one side, an increased elimination of water and chlorides, and an increase, though of lesser degree, in that of total solids, nitrogen and titratable acid. These findings are in accord with those previously noted (3). He attempts to distinguish between section of the splanchnic nerve, section of the renal nerves, and simultaneous section of the vagus and splanchnic nerve in their quantitative effects mainly on the elimination of water. His conclusions, however, do not appear to be justified by an examination of protocols of his experiments; nor are they borne out by the previous work of Marshall and Kolls, whose paper he appears not to have seen. The criticism which he makes of Yoshimura's work explaining the results of nerve section as due to the increased blood flow through the kidney, would apply, if justified, to the previous work of Marshall and Kolls. His criticism is as follows: "Auch die Methode Yoshimuras bringt keine Klarheit in diese Frage, da er durch Abklemmung der einen Nierenarterie in der Blutzufuhr beider Nieren verschiedene Verhältnisse schafft." The indirect evidence obtained with the oncometer by Cohnheim and Roy (12), many years ago, showed that ligation of one renal artery did not increase the blood flow through the other kidney; and even if this is not so, we fail to see how it would effect positive results obtained by the method used by Marshall and Kolls and subsequently by Yoshimura, although negative results would not be conclusive under these conditions.

It remains to discuss the bearing of the results which have been obtained on the theories of the formation of urine by the kidney. The old controversy between the theories of Ludwig and Bowman-Heidenhain is too well known to need mention, and the older literature and evidence has been frequently reviewed and recently summarized by Cushny (13) in his monograph. The so-called Bowman-Heidenhain theory of "vital secretion" is stated so indefinitely and is so compatible with almost any results that it does not permit of experimental testing. The only definite statement which is made by this theory is that the water and inorganic salts are secreted by the glomeruli, while urea and related organic bodies are eliminated by the activity of the epithelial cells of the tubules. The results under discussion indicate clearly that

water and all the inorganic salts cannot be classed together, but that sulphates and phosphates resemble urea more in their behavior than water and chlorides, while ammonia (and presumably ammonium salts) differs from both of these groups, and resembles creatinine in its response to increased blood flow through the kidney. The evidence, however, which has been slowly accumulating points to filtration and its necessary consequence, reabsorption, as processes occurring in the kidney. The recent work of Richards and Plant (14) showing that it is the pressure rather than the volume flow of blood which determines the rate of urine flow from the kidney, would appear to be strong additional support for filtration. Moreover, these investigators have clearly shown that Heidenhain's objection to glomerular filtration because compression of the renal vein diminished urine flow is not justified. The opposite experiment that clamping the renal artery for a minute or two stops the elimination of urine for a long time after the artery is released, has also been urged against filtration. This is discussed in another place by Marshall and Crane (15), and is shown not to be opposed to what might be expected on a filtration theory. For a further recent discussion of the evidence for and against filtration we must refer the reader to Cushny's monograph (13) and the recent Harvey lecture by Richards (16). It would appear then difficult to construct a satisfactory working hypothesis of urinary secretion which did not include filtration and reabsorption as processes taking place in the formation of urine. The first question which then should be answered is whether filtration and reabsorption alone are sufficient to explain all the facts which have been established in connection with renal function, or whether another process or processes are occurring along with filtration and reabsorption.[2]

Cushny has outlined the "modern theory" which he believes will explain the formation of urine on filtration and reabsorption alone. The great advance which has been made in the statement of this theory would appear to be the exactness with which the hypothesis is expressed, and also the possibility, as noted by Cushny, of modifying it to fit new developments. Owing to its definiteness, the "modern theory" admits

[2] We must refer the reader to some of the summaries quoted for a discussion of evidence for secretion by the tubules based almost entirely on histochemical methods of examining the kidney after fixation (and consequent death of the cells). This evidence is not at all conclusive and may be interpreted in other ways. Moreover, it is by no means accepted by many workers in this field. Numerous investigators (17) have proposed theories based on filtration by the glomeruli and reabsorption and secretion by the tubules, but have not shown that secretion is necessary in addition to reabsorption and filtration.

easily of experimental testing. The "modern theory" assumes the passage of a colloid free filtrate of plasma through the glomeruli and the reabsorption of water and certain constituents in constant concentration by the tubules. Substances which pass back to the blood and lymph through the tubules are designated threshold bodies, while those which are not reabsorbed are the no-threshold substances. If an increase in urine flow occurs from any cause, it is obvious that the no-threshold bodies should all be increased in the same proportion, provided no change has occurred in their relative concentrations in the plasma. In the experiments reported in the present paper, any changes in the composition of the plasma are controlled by using the kidney of the unoperated side as a control. The behavior of creatinine and urea, which are regarded as no-threshold substances, does not bear out the above theory, as was pointed out by one of us in a recent paper on water diuresis (18). Mayrs (19), in a recent paper from Cushny's laboratory, has modified the "modern theory," and regards urea as a threshold substance, which would dispose of the above objection. Creatinine, suphate and phosphate are, however, regarded as not being reabsorbed; and, in fact, Mayrs finds that after injection of these substances they are concentrated to about the same extent by the rabbit's kidney. Ammonia would presumably be regarded as a no-threshold substance in the above hypothesis. Our results, which are presented here, indicate clearly that in the dog, creatinine and ammonia differ markedly in their response to increased uninary output from sulphates and phosphates.

The obvious modification of the theory as outlined by Mayrs, would be be to regard sulphates and phsophates as being reabsorbed since they resemble urea closely in their behavior in our experiments. Since creatinine, ammonia and phenolsuphonephthalein are not increased on the side with the greater output of urine, it is quite evident that the amount of glomerular filtrate from this kidney cannot greatly exceed that of the other kidney. The increase might be within the normal limits of creatinine variation of about 10 per cent. But creatinine is frequently slightly decreased on the side with the greater urine flow as may be seen from an examination of the results in this paper and a preceding one of this series (3). On this modification of the simple filtration-reabsorption theory, then, the increased flow of urine after section of the splanchnic nerve must be brought about mainly by decreased reabsorption and not increased filtration. This would be opposed to the general belief that dilatation of the renal vessels increases the glomerular capillary pressure to a sufficient extent to increase filtration. However, since the magnitude

of the glomerular pressure is an unknown factor, the effect of increases in this pressure on the amount of filtration taking place must remain unknown. If the glomerular pressure is really in the neighborhood of the pressure in the renal artery, as is frequently assumed (20), then vasodilatation in the kidney might conceivably increase the effective filtration pressure only to an insignificant extent. The assumption that the diuresis due to increased blood flow through the kidney is due to decreased reabsorption brought about by unknown factors removes the simplicity from the theory that urine formation can be explained by filtration and reabsorption alone. Other more direct evidence, however, indicates that the above explanation cannot be correct.

The recent work of Nash and Benedict (21) and Behre and Benedict (22) clearly indicates that the ammonia and creatinine of the urine cannot be entirely accounted for on filtration through the glomeruli of a protein-free filtrate, for these investigators present evidence to show that the amounts of ammonia and creatinine in the blood are insufficient to account for the amounts eliminated in the urine. Moreover, in the case of ammonia, evidence is presented that the ammonia of the urine is formed in the kidney, and the same explanation is suggested for creatinine. Marshall and Vickers (23) have recently found that phenolsulphonephthalein is present in the plasma in both free and bound forms, that is, that only a certain fraction of the total substance present can be recovered by ultrafiltration. The amount of free phenolsulphonephthalein passing through the kidneys in a given time has been found to be less than the amount eliminated in the urine in the same time. Hence, filtration of even all the plasma brought to the glomeruli cannot alone account for the elimination of this substance, and some other type of process is necessary to remove the bound phthalein from the plasma or to release any which may have been previously stored in the renal cells. After injection, phthalein has been shown to be present in the kidney in a much greater concentration than the blood or other tissues even if the secretion of urine has been prevented throughout the experiment.

The above results would appear to offer an explanation for the constancy of the elimination of creatinine, ammonia and phenolsulphonephthalein with varying urine flow, and make an addition of two processes, synthesis and "secretion," necesssary to the theory of filtration and reabsorption. Substances like urea, phosphates and sulphates may also be eliminated partly by "secretion" as well as filtration, while there would seem no reason to assume the secretion of

water, chlorides or carbonates. Evidence on these questions will have to be obtained by other methods. The explanation which we believe fits in most satisfactorily with these results as well as with the whole mass of data which has been accumulated by numerous workers on urinary secretion is as follows. The passage of the non-colloid constituents of the plasma takes place through the glomerulus by filtration. Water, chlorides, carbonates, (possible urea, sulphates and phosphates) are reabsorbed during the passage of the filtrate along the tubules. Phenolsulphonephthalein (possibly also urea, sulphates and phosphates) are added to the glomerular filtrate by secretion by certain parts of the tubule, while creatinine and ammonia are added by the tubules after being formed in the renal cells. The above description is confined to the substances with which we have worked in this series of investigations and is only used to represent definite classes of substances.[3] Certain others can readily be placed—sodium and glucose along with chlorides and carbonates, while others again such as potassium, calcium, magnesium and uric acid have been insufficiently studied to allow of classification.

SUMMARY

Previous work on the difference in behavior of water, chlorides, urea and creatinine to increased blood flow through the kidney brought about by section of the splanchnic nerve has been confirmed and extended to include the behavior of carbonates, sulphates, phosphates and ammonia. Increased blood flow through the kidney increases markedly the elimination of water, chlorides and carbonates, to a less extent that of urea, phosphates and sulphates, while the elimination of creatinine, ammonia and phenolsulphonephthalein is unchanged under the same conditions. The bearing of the results on the mechanism of urinary secretion is discussed, and it is pointed out that they agree in the most satisfactory manner with a theory of filtration through the glomeruli, and reabsorption and secretion by the tubules. Just what part each urinary constituent bears to these three processes is considered in the light of the information at present available.

[3] It is obvious that phenolsulphonephthalein is not normally eliminated by the kidney because it is not a normal constituent of the body, but it seems equally obvious that the mechanism of elimination of this substance must be shared by some of the normal urinary constituents.

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THERMO-ELECTRIC STUDIES OF TEMPERATURE VARIATIONS IN ANIMAL TISSUES

I. General Considerations; Description of Apparatus and Technique

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Received for publication July 20, 1922

In previous publications (1), (2) we have demonstrated that the intracellular changes in excitation and exhaustion which in certain organs and tissues—notably the brain—are revealed by the microscope, are manifested also by alterations in electrical conductivity.

Histologic studies and measurements of electrical conductivity must be prosecuted after the death of the animal, although, as noted in a preceding report (2), with proper precautions it is reasonable to believe that the changes noted at least parallel the progress of processes in the living animal. However, to determine finally whether or not the variations in the histologic picture and in the electric conductivity are parts of one and the same process as that manifested by changes in functional activity, it was necessary to devise some method by which the functional changes in the cells might be measured in the living animal. Only by such a measure could it be ascertained whether or not changes in electric conductivity indicate changes in functional activity. Clinical evidence would appear to demonstrate that this is the case, but clinical evidence cannot be accurately measured, nor does it identify without question the organs which are principally concerned. Some accurate index that could be applied to the organ itself in the living animal was essential.

Variations in functional activity indicate variations in oxidation; variations in oxidation are manifested by variations in heat production. If heat is a constant product of functional activity, then, if we could measure the progressive changes in the temperature of the various tissues and organs during the various phases of excitation and exhaustion under conditions identical with those formerly studied, we not only

should be able to check our findings in the previous researches but should be able finally to link those findings with the clinical evidence.

As the first means to this end we decided to use the method of measurement employed by physicists for the measurement of minute temperature variations, that is, to employ thermocouples so constructed that they could be applied to the brain, liver, muscle or other tissue of the living animal.

This method of measuring variations in the temperature of living tissues was first utilized, as far as we have been able to discover, by Becquerel and Breschet (3) in 1835, when they used thermocouples to determine variations in temperature in different parts of the body in different pathological conditions. They inserted thermocouples into various muscles of the arm, the thigh and the leg, into the abdomen and into different portions of tumorous growths. They even inserted the couples into the auricles of the heart to determine the difference between the temperature of arterial and venous blood. Their reasons for undertaking this research are significant. In their *Premier Memoire sur la Chaleur Animale*, they state in brief that this research was suggested as the result of certain other attempts to answer the following questions: "Are the vital forces of an electrical or chemical nature? Has the organism its own peculiar mode of action?"

In 1869 and 1870 Schiff (4) published the results of an extensive research undertaken in the hope of answering the following questions:

> Is the stimulation of the nerves of sensation necessarily transmitted to the cerebral hemispheres, or is the direct transmission of the stimulation in the normal animal arrested at the spine or in the pons Varolii? Furthermore, is the transmission to the brain in accordance with the fundamental laws governing transmission along the nerves? Is the formation of a perception in the brain accompanied by phenomena which the means of investigation at our command will not permit us to regard as subject to the general laws of material movement?

In Schiff's research thermocouples were employed which were applied directly to exposed nerves or inserted into different parts of the brain. The author felt that he had established the following points:

1. That "The irritation of the nerve increases its temperature."
2. That successive irritations produced diminished response of the nerve.
3. That every peripheral irritation gave a response in the brain manifested by increased temperature.
4. That the temperature was always higher in the right hemisphere of the cerebrum.

5. That there was no temperature change in the cerebellum.

6. That no response to peripheral irritation was noted in the brains of animals under morphin. "These preparations (of opium) especially of morphin do not permit any manifestation to an appreciable degree of the effect of sensory irritation on the temperature of the brain."

7. That not only tactile sensation but stimulation of all the organs of special sense produced an increase in the temperature of the cerebrum.

8. That repeated excitation of the special senses—sight, hearing—produced progressively lessening effects as manifested by temperature changes in the brain. "It is seen, consequently, that this last series of experiments is perhaps the most important for our conclusions."

9. That "Psychic excitation, independent of the sensations which produce it, is accompanied by a production of heat in the nerve centers, which is quantitatively greater than the heat engendered by less complex sensations."

The author believed that his observations definitely excluded the possibility that the temperature changes noted were due to circulatory changes; and that his experiments were sufficient "to establish, with all the desirable precision, that the production of heat which we have observed is really the result of excitation which is peculiar to and an intrinsic part of the nerve elements."

In 1868 Rosetti (5) recorded an attempt to construct thermocouples for the measurement of body temperature and reported one practical application.

Also in 1868, Lombard (6) described a thermopile devised by him for the study of external temperature changes. In particular he studied the relation of heat to mental work as manifested by temperature changes of the outside of the head, which he believed could be referred to changes in the temperature of brain. Albutt (7) in 1875 employed similar apparatus to that of Lombard in an attempt to discover the relation between internal and external heat.

From that time the literature records the occasional use of thermo-electric apparatus for the measurement of changes in bodily heat, but no especially noteworthy reseaches are recorded until within recent years. A bibliography of these intermediate studies is offered by Benedict and Snell (8) in their description of a resistance thermometer devised by them for the observation of variations in the rectal temperature of subjects in a calorimeter chamber.

In 1909 Gamgee (9) devised a clinical apparatus in which thermocouples were employed for the continued measurement and registration

of diurnal variations in temperature, and following his lead, Sims, Woodhead and Varrier Jones (10) produced a thermo-electric apparatus which could be used consecutively for 24 hours or more without disturbing the patient.

In 1911, A. V. Hill (11) utilized the thermo-electric method in an investigation to determine the presence or absence of temperature changes during the transmission of a nervous impulse. He concludes that

> Tetanus, up to 25 secs., of a live nerve, does not cause any change of temperature (other than at the seat of excitation) of more than about $\pm 6 \times 10^{-6}$°C. There is no evidence of any change at all, but the method does not allow conclusions beyond this limit. For every single propagated disturbance, the change of temperature, therefore, cannot exceed about 10^{-8}°C., a hundred millionth of a degree. This corresponds to an oxidation process in which only one molecule of oxygen is used in a space of visible size, viz.,—a 37μ cube. This suggests very strongly, though of course it does not finally prove, that the propagated nervous impulse is not a wave of irreversible chemical breakdown, but a reversible change of a purely physical nature.

A thorough search of the literature, however, has discovered no reference to the measurement of variations in the temperature of the brain and other internal organs under diverse conditions with the exception of the work of the investigators cited above, that of Stengel and Hopkins (12) on the intragastric temperature and the work of Macleod and Taylor (13) on the effects of hot and cold applications on the superficial and deep temperatures.

By the invitation of Dr. E. P. Hyde and with the active coöperation of Dr. W. E. Forsythe, our initial experiments were performed at the Nela Research Laboratory. A copper-constantan thermocouple, the wires passed through a glass tube in the end of which the couple was sealed, was used, the "cold" junction being placed in melting ice in a thermos bottle.

Three rabbits were used and as the purpose of this test was only to determine the feasibility of proceeding further, a series of rapid tests was made with most gratifying and encouraging results. This preliminary experiment proved:

1. That it is possible to insert a thermocouple directly into the brain, the liver, the muscle, the pleural cavity, for a prolonged period without any notable effect upon the general condition of the animal.

2. That practically any alteration in the condition of the animal—light and deep anesthesia, struggling, shock-producing manipulations,

etc.—is accompanied by a measurable change in the temperature of the brain.

3. That the temperature of the brain decreases progressively under ether anesthesia.

4. That the injection of adrenalin produced a characteristic rise and fall in the temperature of the brain closely related to the clinical phenomena,—a finding of extreme significance in view of our later studies.

These curves were not plotted, nor was any attempt made to translate the galvanometer readings into actual temperature variations, but the purpose of this preliminary experiment having been attained, we proceeded to devise the essential apparatus for an extensive investigation. We wish at this point again to express our appreciation to Doctor Hyde and Doctor Forsythe for their interest and active coöperation in securing this first evidence.

Apparatus: The essential apparatus consisted of Leeds and Northrup galvanometers of types R and H with especially constructed copper-constantan thermocouples made of 5 mil wire twisted together and soldered. The recording junction was exposed, the leads being separated from each other and protected by concentric glass tubes, the ends of which were joined by dental cement. The junction was kept as small as possible and the protecting glass tubes were of the smallest possible caliber. These tubes were bent into such shapes as could be most easily and firmly secured in the tissue for which each was designed. This is a vitally important point, as closeness and constancy of contact of the thermo-junction with the tissue under examination is essential to the attainment of dependable records.

The use of any metal in the construction of the thermocouples was avoided, for fear it might conduct the heat away from the area of insertion and lead to local cooling. This is an essential precaution in working with an instrument so sensitive to small temperature changes; moreover, it is very important to remove any material which would produce a lag in the response of the indicating instrument to the local changes of temperature.

The "cold" or constant junction of the thermocouple was immersed in a tube of oil suspended in a constant temperature bath, the temperature of which was maintained at 39°C., thus bringing the whole range of temperature in the tissues studied upon the scale of the galvanometers. As the extreme range of temperatures involved in living rabbit tissues do not exceed 7°C., it is possible to measure variations by the direct deflections of a galvanometer of suitable sensitivity.

The circuit was of very low resistance with all wire connections soldered and symmetrical, and with all contacts non-frictional and of low resistance. The galvanometers were short-circuited when not in use and were protected from extreme deviations.

Calibrations were made immediately after the measurements to which they applied, as there is always a tendency for the instruments to drift from day to day. The calibrations were made by immersing the active junctions along with a standard thermometer in water in a thermos bottle. To avoid error due to the lag in the response of the large bulk of mercury in the standard mercury thermometer, the readings were always made on a falling temperature, as the decrease was so gradual as to minimize error. With this apparatus, temperature variations could be measured to within 0.01°C.

Technique: As far as possible in each group of experiments rabbits of approximately equal size and age were used. This point was not as essential, however, as in our conductivity studies—as in these experiments each animal acted as its own control.

A small hole through the skull over the cortex at the right of the median ridge was made by an especially constructed trephine, the size of the hole being just large enough to admit the glass tube containing the thermo-junction. This tube was bent at an angle, one arm of which was just long enough to enter the brain to a depth of approximately one-third to one-half a centimeter, the other lying along the top of the head. Absorbent cotton was placed over this to eliminate the chance of chilling, the whole being firmly secured in place by strips of adhesive. By this means the thermo-junction was held securely in place even when the animal moved violently.

It was more difficult to secure an immovable junction in the liver—but in most instances this was accomplished by inserting one arm of a right-angled tube through a small opening just below the ribs—and securing it by metal clips through the muscle, with adhesive strips over the skin. By bending the tubes at a right or slightly acute angle, and the use of clips and abundant adhesive, constant contact could usually be secured in any tissue and by the free use of cotton, complete protection from external chilling was assured.

In the early experiments only one galvanometer was used. As this precluded the possibility of simultaneous readings of the variations in temperature in different organs, another galvanometer was installed. Thus, two observers could make synchronous readings at 15-second intervals or less.

Seventy-seven rabbits were used in these preliminary studies, consistent records being secured in sixty-three. Any experiment was discarded if at the termination of the experiment any fault in contact was found, the insertion of the thermo-junction being examined at the conclusion of every experiment. As noted above, calibration at the conclusion of each group of experiments and repeated testing of the constant temperature checked the findings throughout.

In these preliminary studies, the effects of various agents and procedures has been observed, the temperature variations in the brain being measured in every instance, in the liver in most of the animals, and in the muscle and thyroid each in two instances.

In the first experiments, as noted above, the galvanometer readings were recorded at 30-second intervals, readings being made alternately when more than one thermocouple was employed. Later by the use of two galvanometers and two observers simultaneous readings were made at 15-second intervals.

Fifteen-second intervals, however, are not sufficiently short to assure the recording of every variation in the temperature of the brain. Frequently a rapid deviation of the galvanometer was observed between the recorded readings, but to assure the greatest possible accuracy by the undivided attention of the observers to the 15-second readings, they were told to allow no other observation to interfere with the correct recording of these—an important injunction in experiments which in many instances were $1\frac{1}{2}$ to 2 hours or more in length, requiring the accurate reading and recording of 300 and more galvanometer readings by each observer. It early became apparent that a method of continuous record is required to register all the temperature changes in the infinitely sensitive brain, for the slightest stimulation of the animal under observation—by the sound of a closing door, a motion of the hand before the eyes, a light touch, etc. produced a measurable, if but slight and brief, alteration in the brain temperature. In many instances an abrupt rise was observed immediately prior to a struggle which was marked by a sharp fall. This was observed so often that when in a quiet animal the temperature of the brain rose abruptly, a struggle was expected.

The variation in the temperature of the brain which preceded and accompanied a struggle occurred in such rapid succession that it was impossible in every instance to synchronize the event and the galvanometer reading with certainty. The apparent variations in the nature of the response to certain stimuli which will be noted in some of the

charts, are undoubtedly due to this difficulty which can only be obviated by continuous records.

In many cases various forms of stimulation were applied to the same animal. In the following presentations, therefore, instead of following the usual method of presentation of protocols, the findings will be summarized according to the various forms of stimulation employed.

Especial appreciation is due to Donald D. Forward for his efficient coöperation in many details of this research and to George Harris Crile for his patient and accurate readings of the galvanometer.

SUMMARY

1. By the use of especially constructed thermocouples it is possible to measure temperature variations in living tissues to within 0.01°C.

2. By the described apparatus and technique the progress of functional changes in the brain and other tissues in stimulation and exhaustion from various causes has been observed, and the findings correlated with the histologic changes and the alterations in electric conductivity established by previous researches.

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THERMO-ELECTRIC STUDIES OF TEMPERATURE VARIATIONS IN ANIMAL TISSUES

II. Effects of Anesthesia; Electrical Stimulation; Abdominal Trauma; Exposure of Viscera; Excision of Organs; Acid; Alkali; Strychnin; Diphtheria Toxin

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Received for publication July 20, 1922

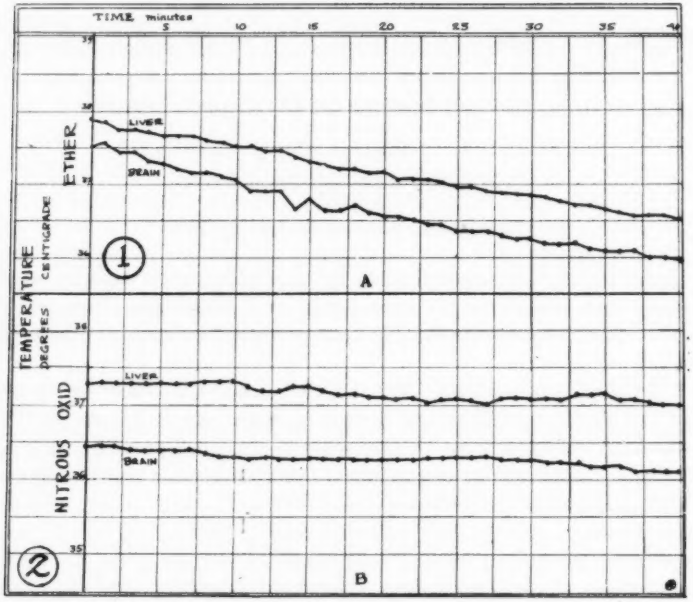
Under the methods described in the preceding section of this report, animals were subjected to stimulation and exhaustion from various agents and the temperature variations produced by each were observed and recorded.

1. Inhalation anesthesia. Inhalation anesthesia—ether or nitrous oxid—produced a continuously progressive decrease in the temperature of the brain and the liver, this decrease being much greater in the case of ether. In the course of prolonged ether anesthesia the temperature of the brain and of the liver may decrease from 1½ to 2° (charts 1 and 5) or as shown in chart 3, the decrease may amount even to 4°; while under even prolonged nitrous oxid oxygen anesthesia, the decrease in temperature, though progressive, is so slight as to be practically negligible (chart 2). The initial stage (stage of excitement) both of ether and nitrous oxid anesthesia is marked by a slight increase in the temperature of the brain (charts 4 and 5). This initial increase in temperature corresponds to the hyperchromatic stage described in our histologic studies of the brain cells and to the increased electric conductivity of the brain which we found was present in the first stage of both ether and nitrous oxid anesthesia.

2. Electrical stimulation. In this group of experiments the current from a single dry cell attached to an induction coil was used, the coil being so adjusted as to produce a sharp tingling of the tongue with the terminals about 0.5 cm. apart. The terminal wires were applied directly to the sciatic nerve. As indicated by the charts (charts 6 to 10), the sciatic nerves on both sides were exposed. On each side stim-

lation was applied first to the unblocked nerve which was then novocainized and the current again applied. The animals were under ether anesthesia throughout.

As shown by the charts, stimulation of the unblocked nerve produced a change in the temperature of both the brain and the liver *in opposite*



Charts 1 and 2. Comparison of the effects of ether and of nitrous oxid anesthesia on the temperature of the brain and liver. (These animals were already under anesthesia when the thermocouples were inserted.)

directions. In certain cases the first stimulation produced an increase in the temperature of the brain and a decrease in the temperature of the liver; while the like stimulation of the opposite nerve produced a decrease in the temperature of the brain and an increase in the temperature of the liver, these opposite effects being especially marked when the first response had been particularly severe (chart 6). After complete blocking of the nerve there was but slight or no response to stimulation of the sciatic nerve.

The similarity of the temperature changes of the brain and the liver resulting from electrical stimulation of the sciatic nerve with those which

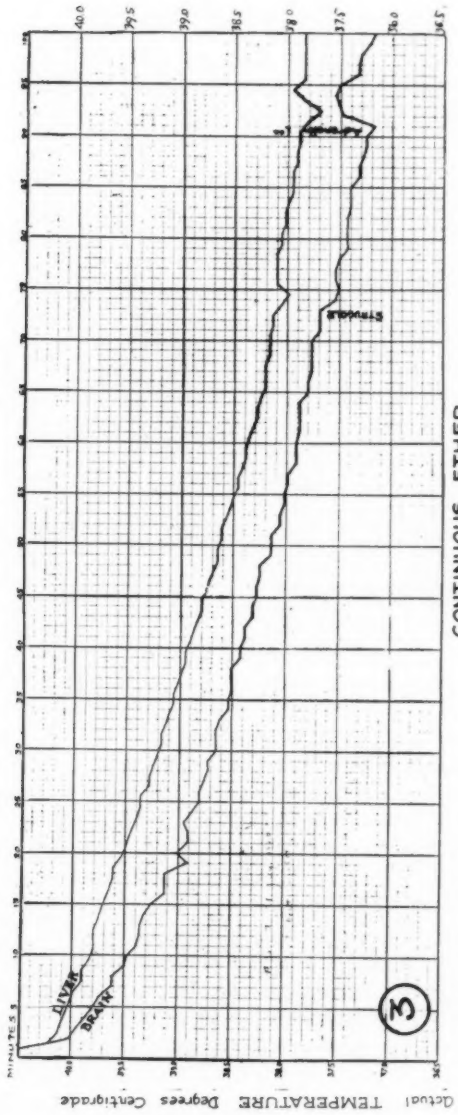


Chart 3. The effect of prolonged ether anesthesia upon the temperature of the brain and the liver. (The animal was already under anesthesia when the thermocouples were inserted.)

accompany a voluntary struggle will be noted by comparing the above cited charts with chart 11.

3. Abdominal trauma, exposure of viscera, etc. Every injury of the abdominal wall or shock-producing manipulation of the peritoneum or intestine was registered by an alteration in the temperature of the brain.

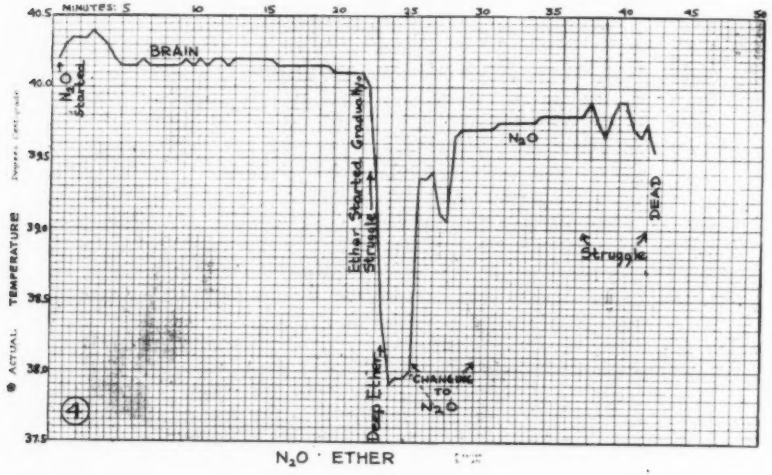


Chart 4. Comparative effects of ether and of nitrous oxid anesthesia on the temperature of the brain. Note initial increase in temperature.

These variations, as is illustrated in the first portions of the charts which follow, were especially manifested during the manipulations essential to excision of the adrenals or to ligation of the liver.

Exposure of the viscera produced a precipitate decrease in the temperature of the brain (charts 12 to 14). The like fall in the temperature in the liver which in part may be attributed to the direct exposure, may in part be due to other causes.

Hot water in stomach: The introduction of hot water into the stomach through a stomach tube increased the temperature of the brain and the liver, *the increase in the temperature of the brain occurring first.*

In some instances, as shown in the charts, the temperature of the liver did not begin to increase until a minute or more after the beginning of the increase in the brain was noted (charts 5 and 12 to 14).

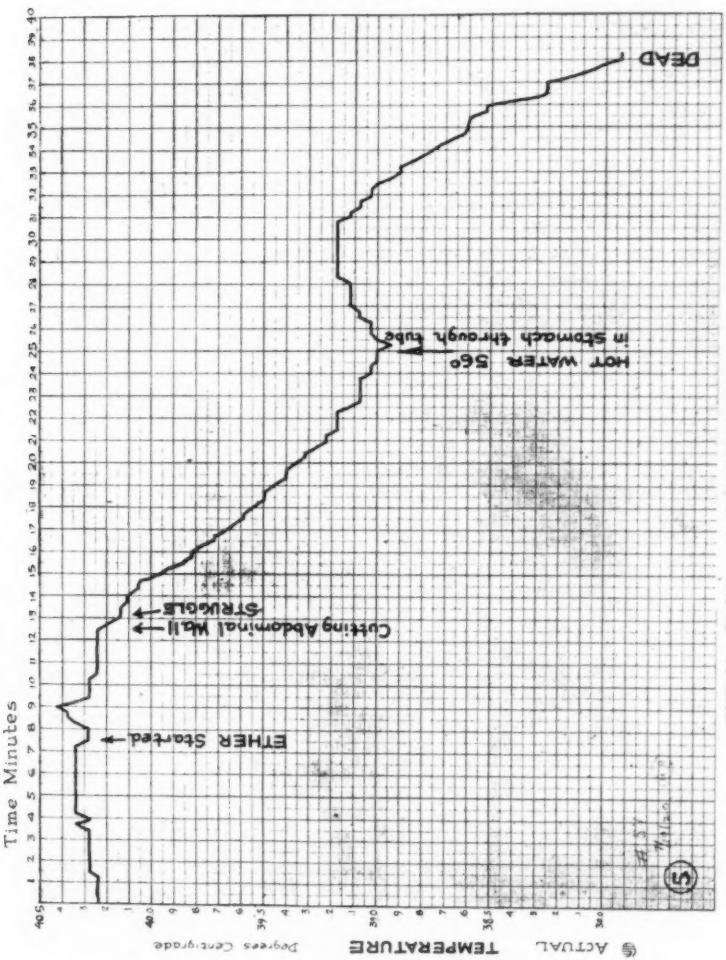
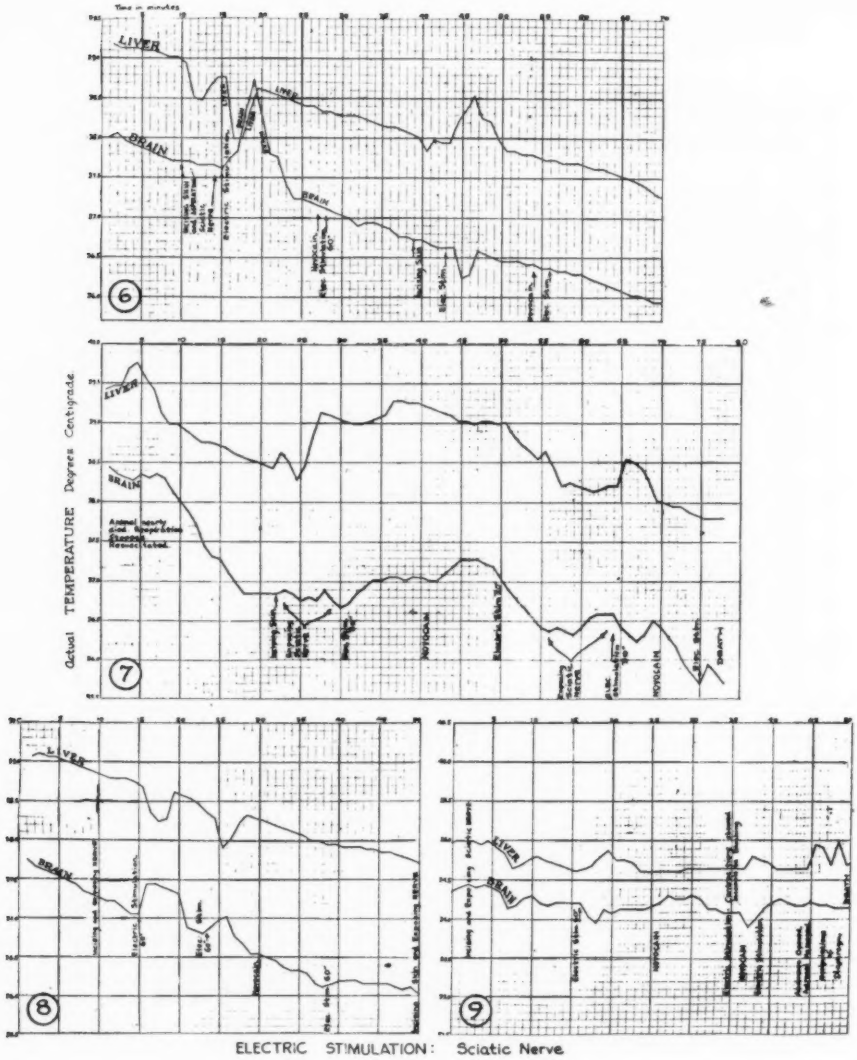


Chart 5. Effect of ether anesthesia upon the temperature of the brain; stimulating effect of hot water in stomach. Note the initial increase in temperature immediately after the administration of ether was started.



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Charts 6 to 9. Effect of electrical stimulation of the sciatic nerve on the temperature of the brain and the liver. Note opposite effects on brain and liver; and that there was little or no change in temperature after the nerve was blocked with novocain.

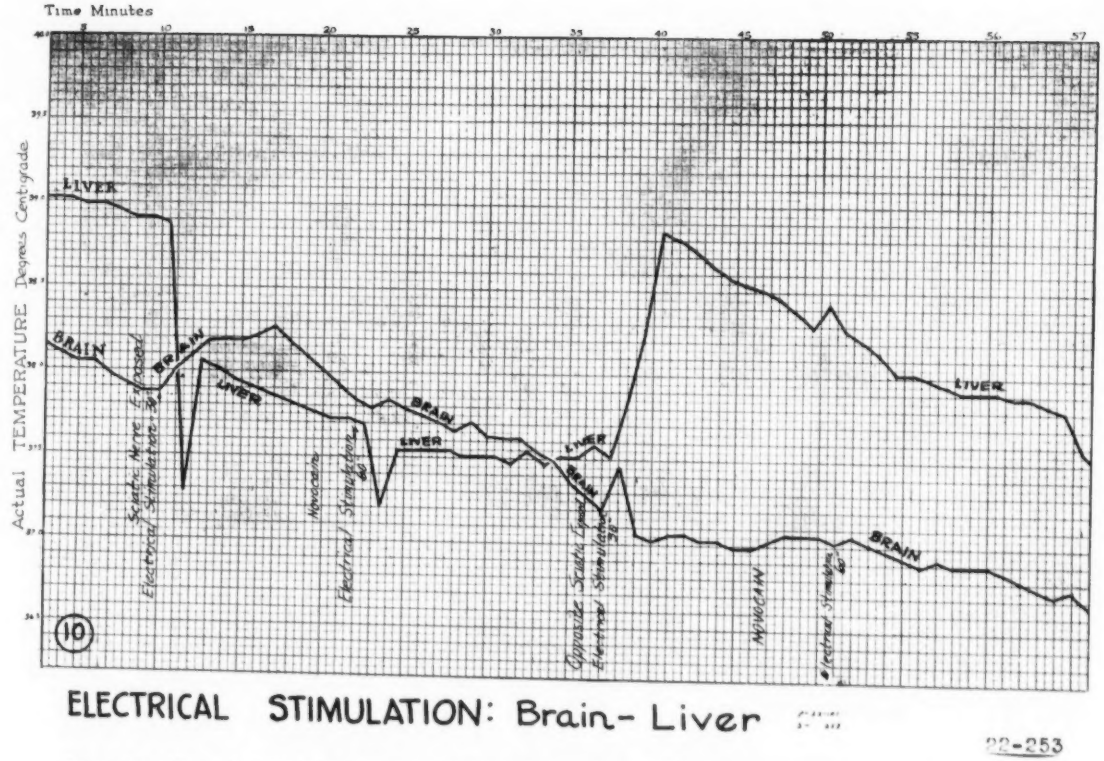


Chart 10. Effect of electrical stimulation of the sciatic nerve on the temperature of the brain and the liver. Note the opposite effects on the brain and the liver and the diminished response of the brain after blocking of the nerve with novocain.

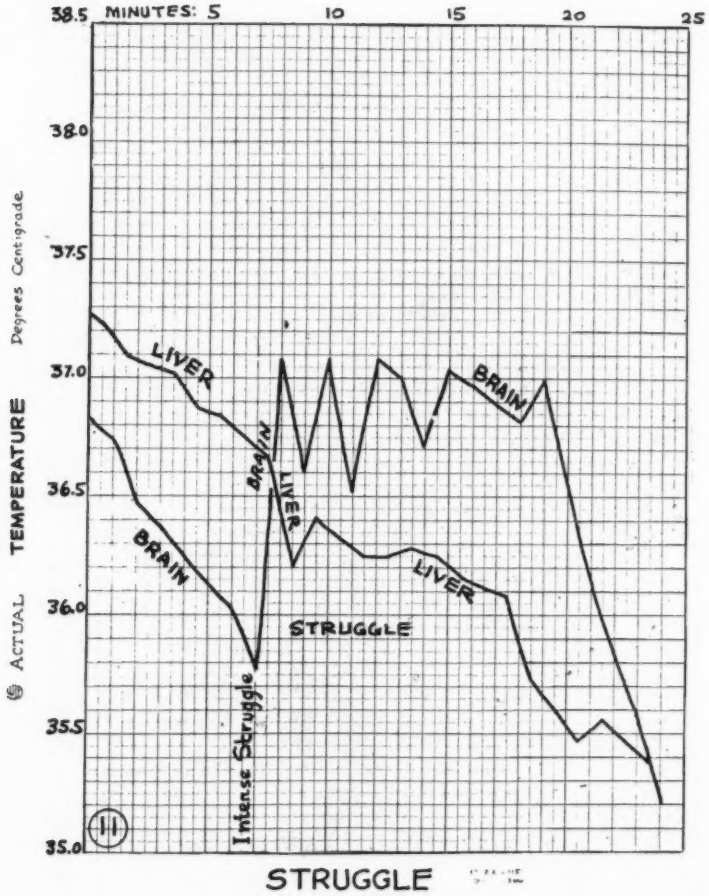


Chart 11. Temperature changes in the brain and the liver during a voluntary struggle. Compare with charts 6 to 10.

4. Excision of organs. *Hepatectomy* was followed by a continually progressive fall in the temperature of the brain which was unchecked by any therapeutic measure. The rapidity of the decrease however varied according to the condition of the animal and the amount of trauma produced by ligation of the liver. Charts 15 and 16 show the typical picture, which is strikingly like that in chart 3 (ether

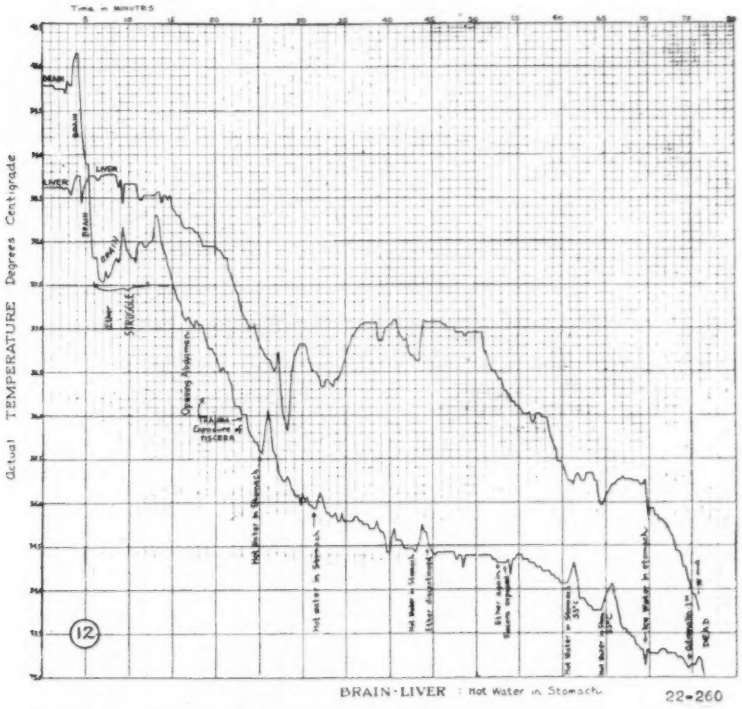
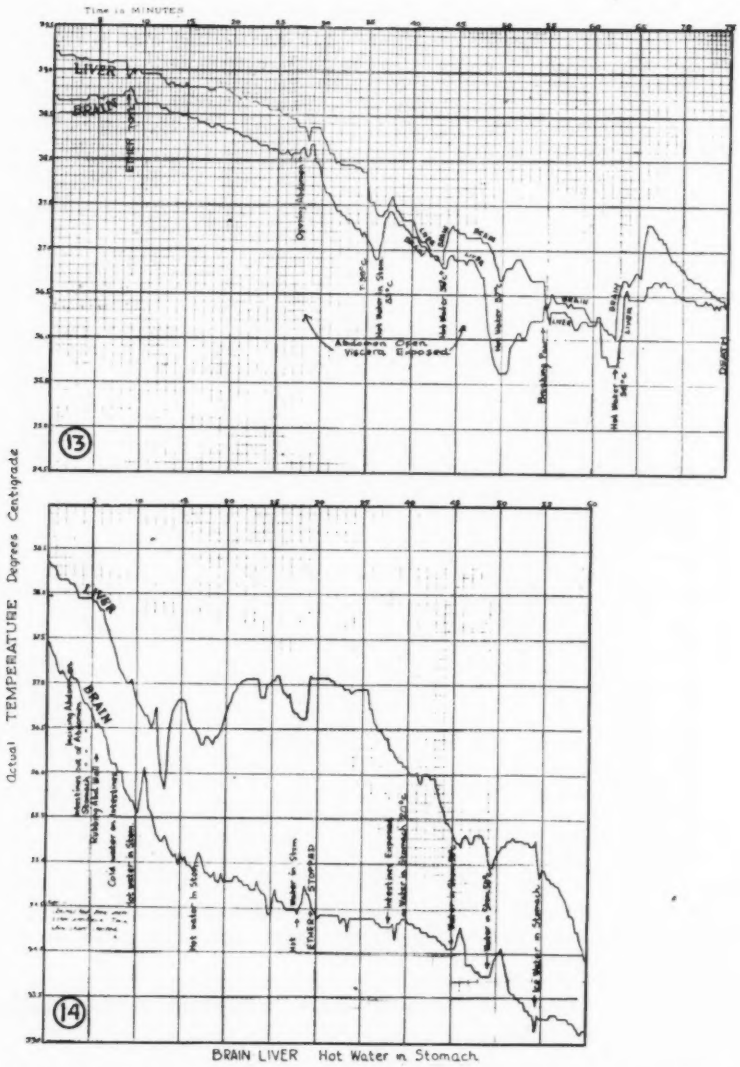


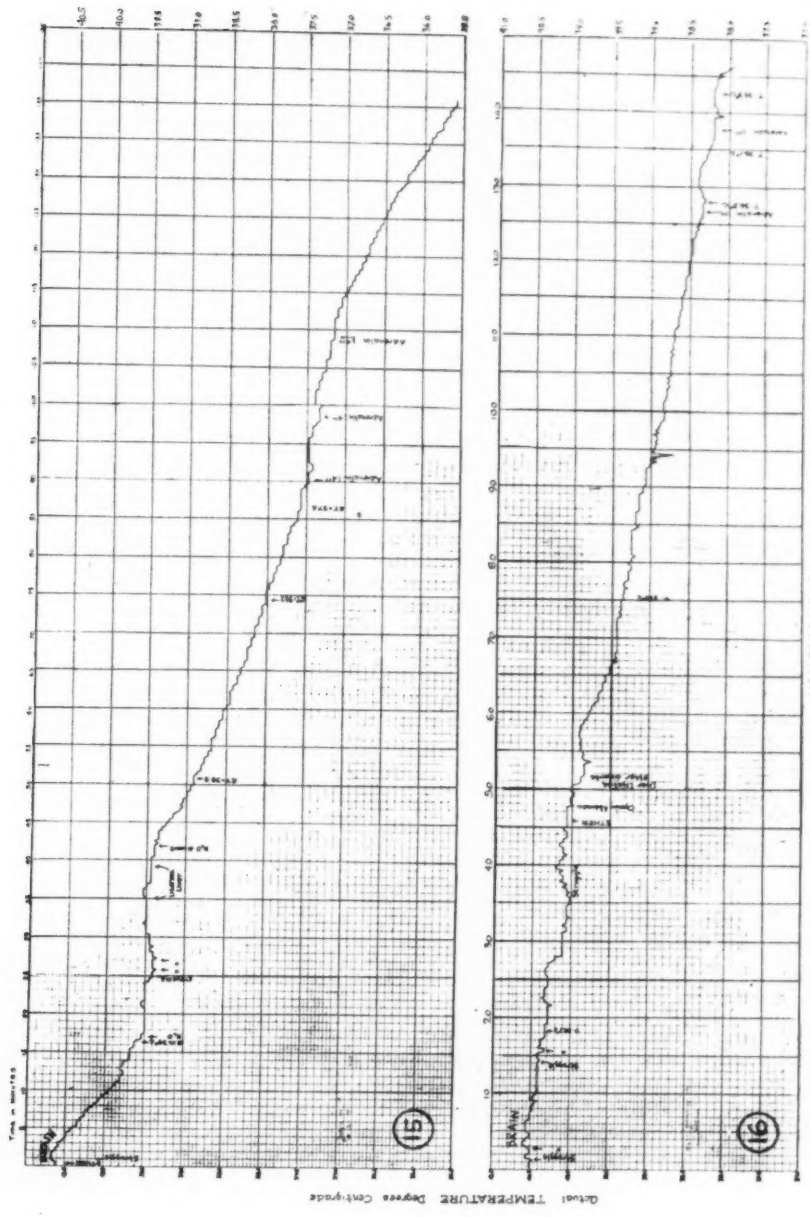
Chart XII. Effects on the temperature of the brain and the liver of abdominal trauma and exposure of viscera and of the introduction of hot water into the stomach.

anesthesia). Chart 17 shows a more precipitate decline in the temperature of the brain.

Adrenalectomy produced a rapid decrease in the temperature of the brain, although the short duration of life in two of the three animals in which adrenalectomy was done makes it impossible to determine



Charts 13 and 14. Effects on the temperature of the brain and the liver of abdominal trauma and exposure of viscera and of the introduction of hot water into the stomach.



Charts 15 and 16. Effect of hepatectomy on the temperature of the brain. Typical results.

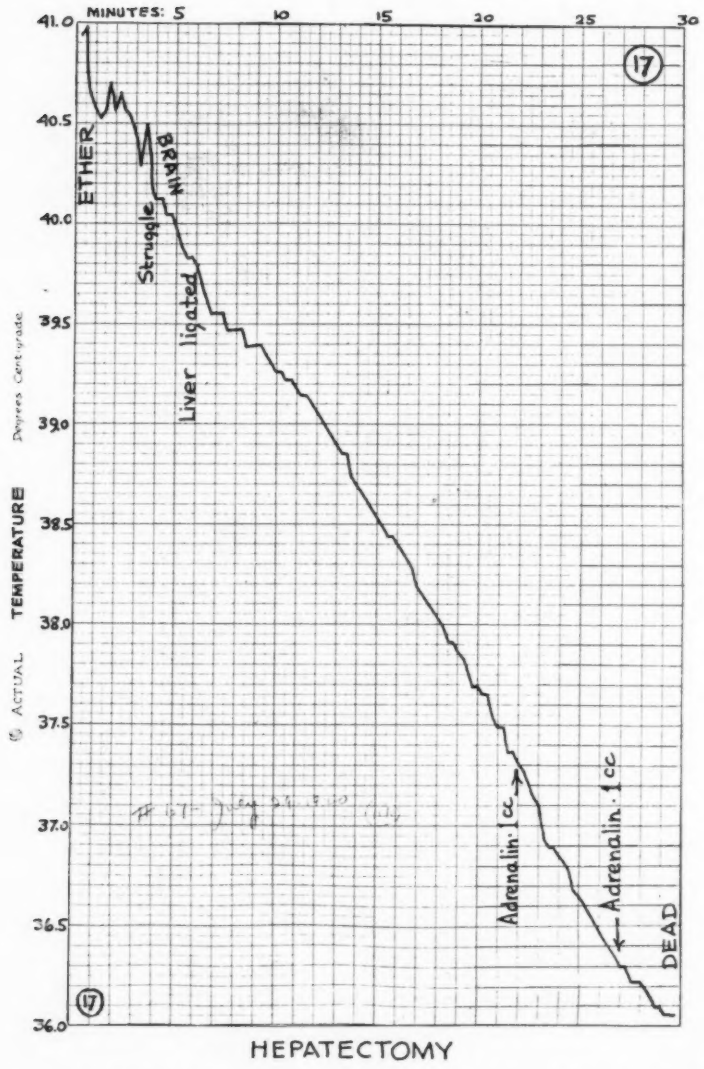


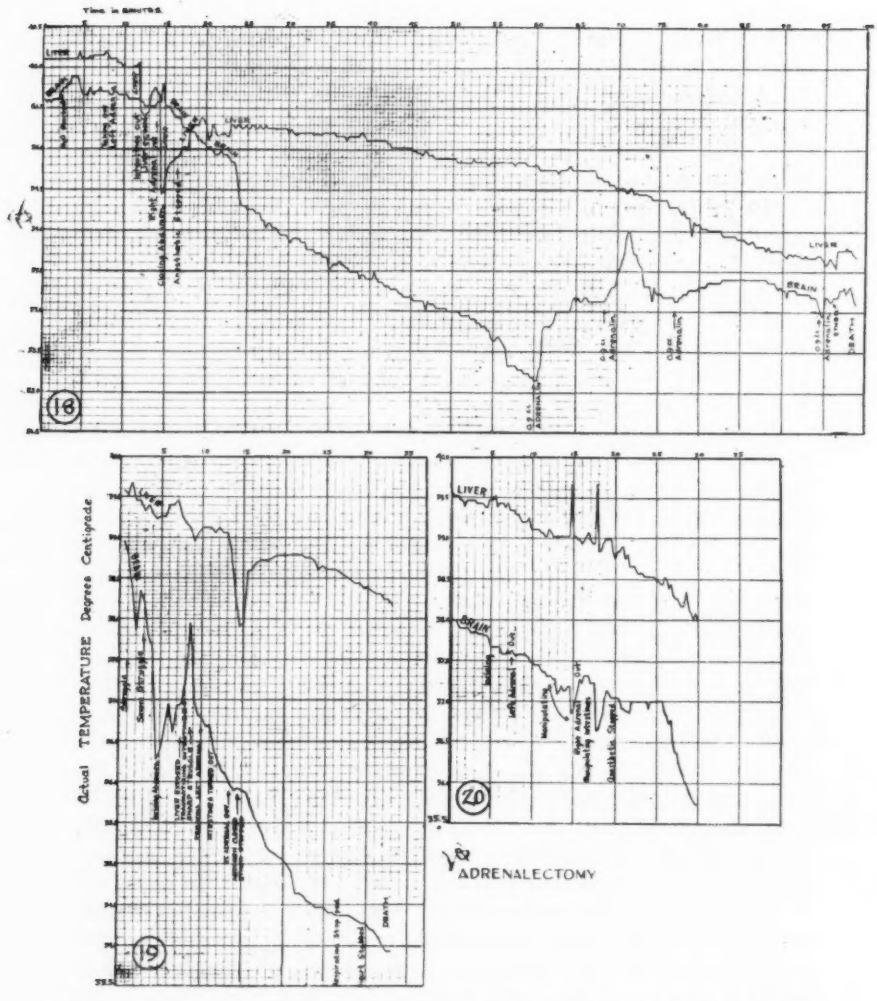
Chart 17. Effect of hepatectomy on the temperature of the brain. An unusually precipitate decline.

to what degree this decrease was due to the trauma of the operation. An animal in which the adrenals were removed with a minimum of manipulation probably presents a typical picture (chart 18). It should be noted in this and in chart 19 that in contrast to the marked change in the temperature of the brain, the temperature of the liver is but slightly altered.

5. Acid. In one experiment the intravenous injection of hydrochloric acid (1 cc. + 10 per cent) was immediately followed by an abrupt rise in the temperature of the brain amounting to 0.7°C. with an equally abrupt fall to 0.56°C. below the point at which the acid was injected; from this point there was a rapid and precipitous decline until death which occurred 5 minutes after the injection. The liver showed no response to the acid injection and at the time of death the temperature of the liver was but 0.46°C. below the point at which the acid was injected, while the total decline in the temperature of the brain amounted to 1.63°C. (chart 21).

6. Alkali. In one experiment 8 cc. of a saturated solution of sodium bicarbonate were injected intravenously. During the injection, which lasted 4 minutes, the temperature of the brain rose 0.5°C. There followed a fall to a point 0.5° below the point at which the injection was started, this period lasting for 8 minutes. During the following 10 minutes there was a further gradual decrease of 0.33°. Ether was then given, the abdomen opened, viscera exposed and extreme shock-producing manipulations were made. Although the fall in brain temperature was accelerated thereby the picture does not correspond to that produced by like exposure and trauma in other experiments. The animal lived for 15 minutes after the abdomen was closed, and was finally sacrificed when the temperature of the brain had reached the low point of 34°C. As after the injection of hydrochloric acid, the *liver* showed no response to the injection of sodium bicarbonate, its temperature declining but slowly until the viscera were exposed (chart 22).

7. Strychnin. The effects of strychnin injection upon the temperature of the brain varied in relation to the clinical effects. In an animal in which typical convulsions occurred there were correspondingly great variations in the brain temperature (chart 24); while in another in which the muscular contractions were less marked the variations in the temperature of the brain were correspondingly less than in the former instance (chart 23). In no instance was the temperature of the liver affected by the injection of strychnin.



Charts 18 to 20. Effects of adrenalectomy on the temperature of the brain and the liver.

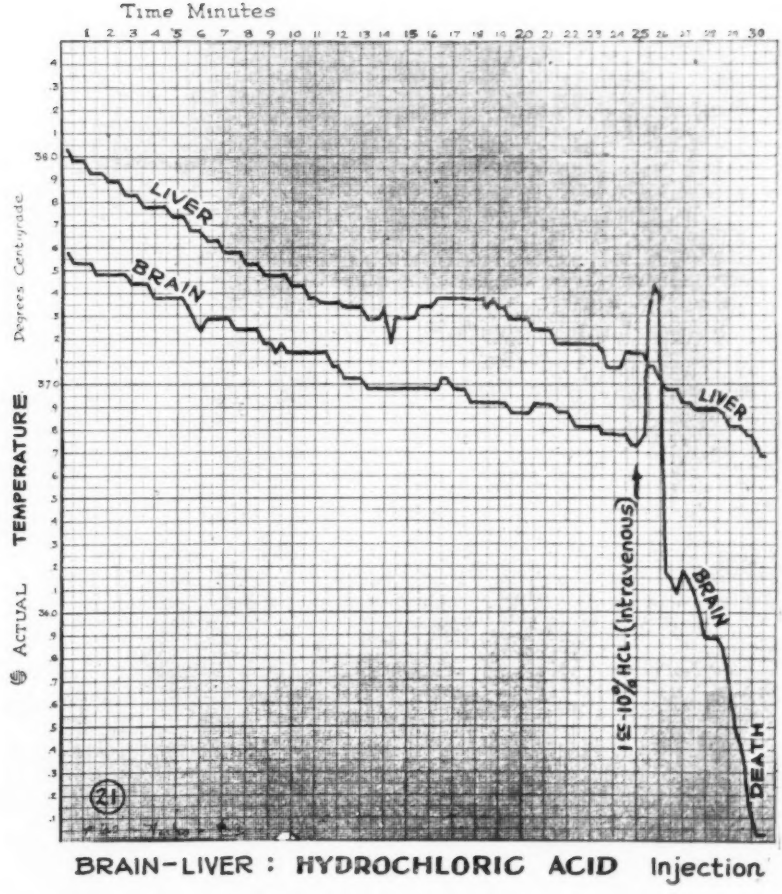


Chart 21. Effect on the temperature of the brain of the intravenous injection of hydrochloric acid. Note the lack of response in the liver.

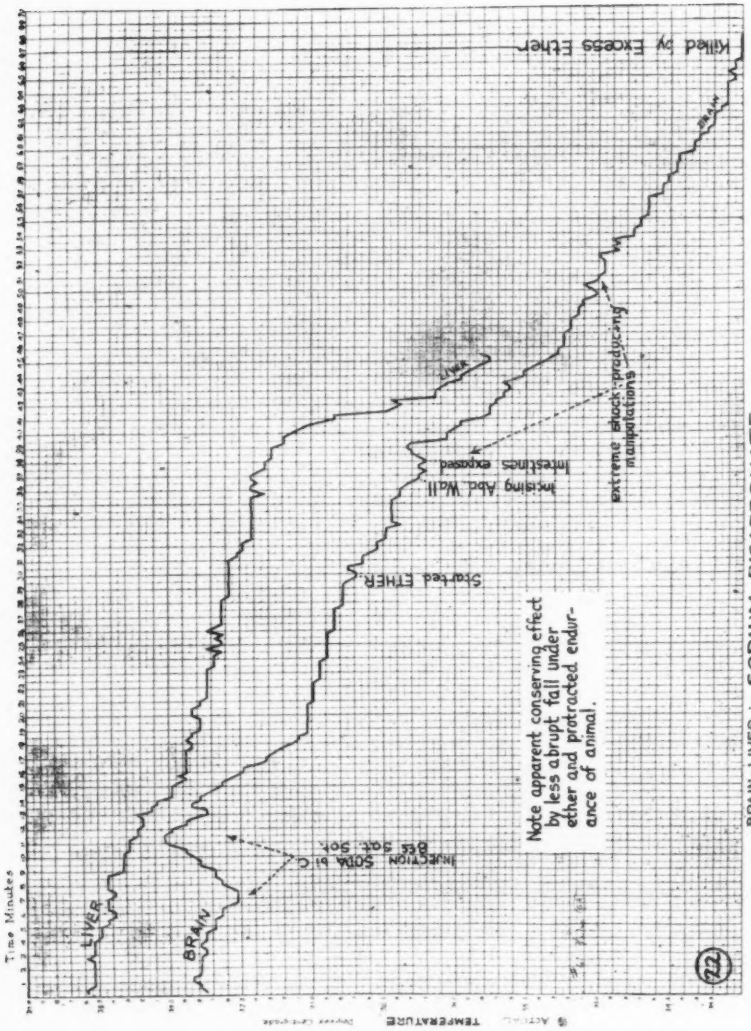


Chart 22. The effect on the temperature of the brain of the intravenous injection of sodium bicarbonate. Note the lack of response in the liver.

In two animals under nitrous oxid anesthesia the injection of strychnin produced but a slight variation in the brain temperature (charts 26 and 27), although in one of these (chart 26) there were strong convulsions.

The effect upon the temperature of the brain of a struggle with convulsive-like movements has already been shown in chart 11, which should be compared with these. Note that in the voluntary struggle, however, the temperature of the liver also was affected.

8. Diphtheria toxin. The temperature of the brain and liver was watched continuously for hours after the injection of diphtheria toxin. No significant change in the liver was noted but there were variations in the temperature of the brain. In one instance, on the day after the injection of the toxin, the animal was placed under ether anesthesia when the brain showed a precipitate decline in temperature as shown in chart 28, death occurring 21 minutes after the anesthesia was started. The rectal temperature when the toxin was injected was 38.5°C. At 8:00 o'clock on the following morning it was 41.1°, and $2\frac{3}{4}$ hours later when this chart was started it had fallen to 40.8°. Eight minutes before death it was 38.8°.

SUMMARY

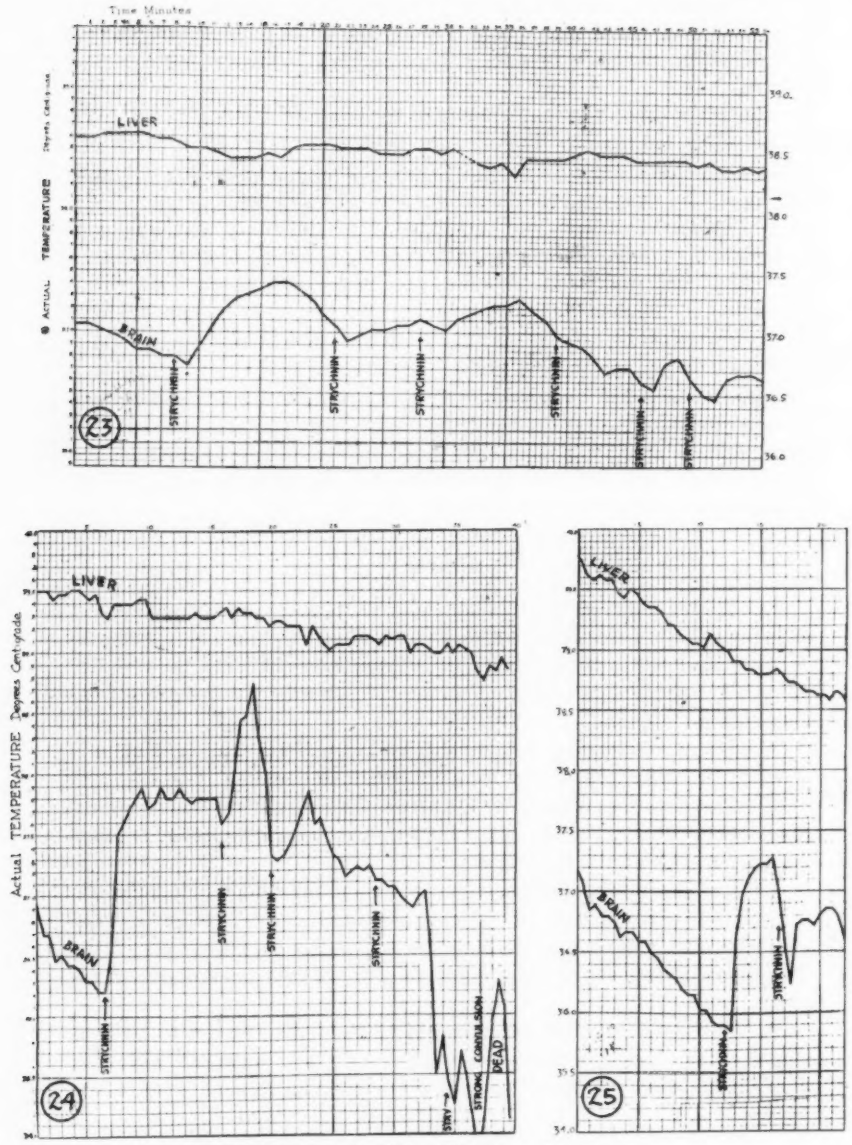
1. The progress of exhaustion from any cause—continuous ether anesthesia, adrenalectomy, physical trauma—is marked by a progressive decrease in the temperature of the brain and the liver, the rapidity of which bears a direct relation to the rate at which the degree of exhaustion advances.

2. The stage of excitement of ether and of nitrous oxid anesthesia is marked by an increase in the temperature of the brain.

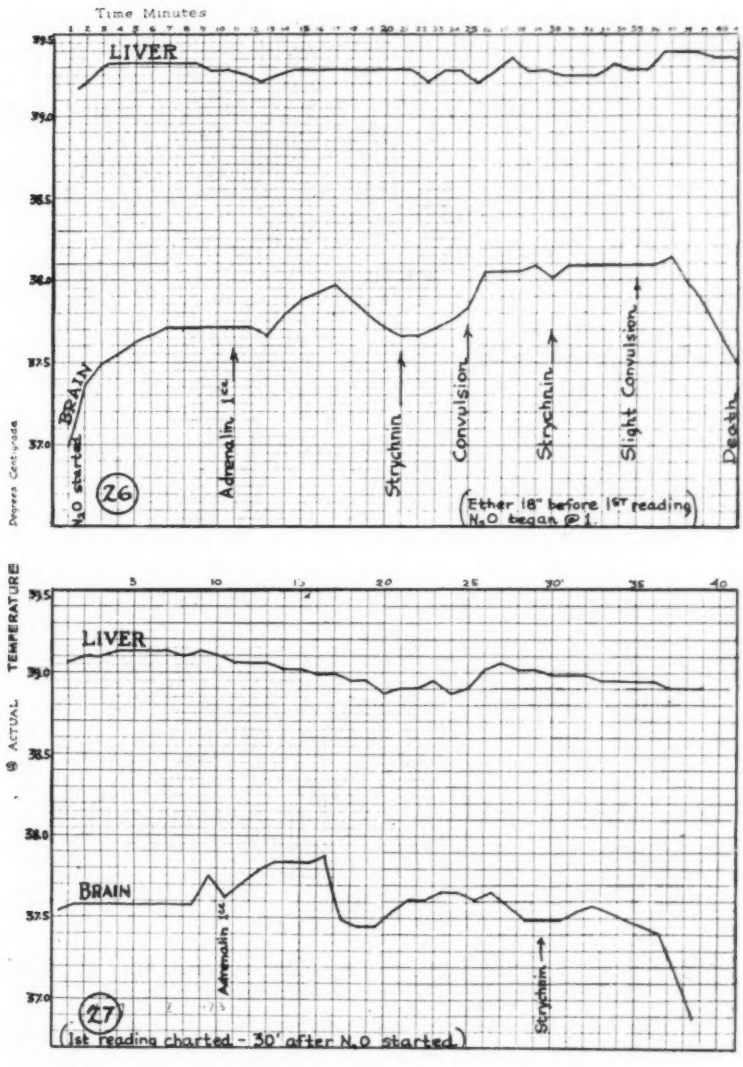
3. After hepatectomy the temperature of the brain declines progressively until death, the resultant curve corresponding closely to that produced by continuous ether anesthesia.

4. Muscular activity, either voluntary or produced by direct electric stimulation of a nerve, is accompanied by rapid alterations in the temperature of the brain and the liver corresponding to the phases of the muscular activity—these alterations however being in opposite directions.

5. No alteration in the temperature of the liver appears to be produced by the injection of strychnin, of an acid or of an alkali, although marked and characteristic changes, corresponding in each case to the



Charts 23 to 25. Effect of strychnin on the temperature of the brain. Note the lack of response in the liver.



NITROUS OXID : ADRENALIN : STRYCHNIN

Charts 26 and 27. Effect of nitrous oxid anesthesia upon the response of the brain to strychnin. Compare with charts 23 to 25.

clinical phenomena, are produced by each in the temperature of the brain.

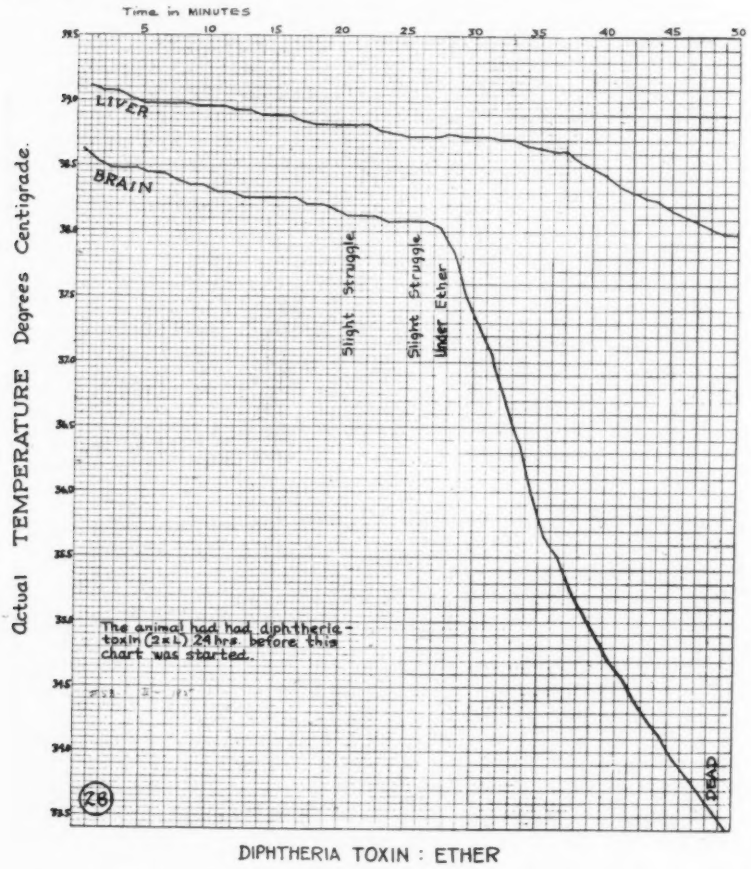


Chart 28. Effect of ether anesthesia upon the temperature of the brain of an animal 24 hours after the injection of diphtheria toxin.

6. Exposure of the viscera and abdominal trauma alike produce a rapid fall in the temperature of the brain and the liver, the change in the latter being in part but not entirely accounted for by the direct chilling of the liver substance.

7. The restorative effect of the introduction of hot water into the stomach is marked by an immediate elevation of the temperature of the brain which measurably precedes—in some instances by a minute or more—the resultant elevation in the temperature of the liver.

8. The lowered resistance to inhalation anesthesia produced by an acute infection is illustrated by the rapid decline in the temperature of the brain of an animal subjected to ether anesthesia 24 hours after an intravenous injection of diphtheria toxin.

THERMO-ELECTRIC STUDIES OF TEMPERATURE VARIATIONS IN ANIMAL TISSUES

III. Adrenalin

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Received for publication July 20, 1922

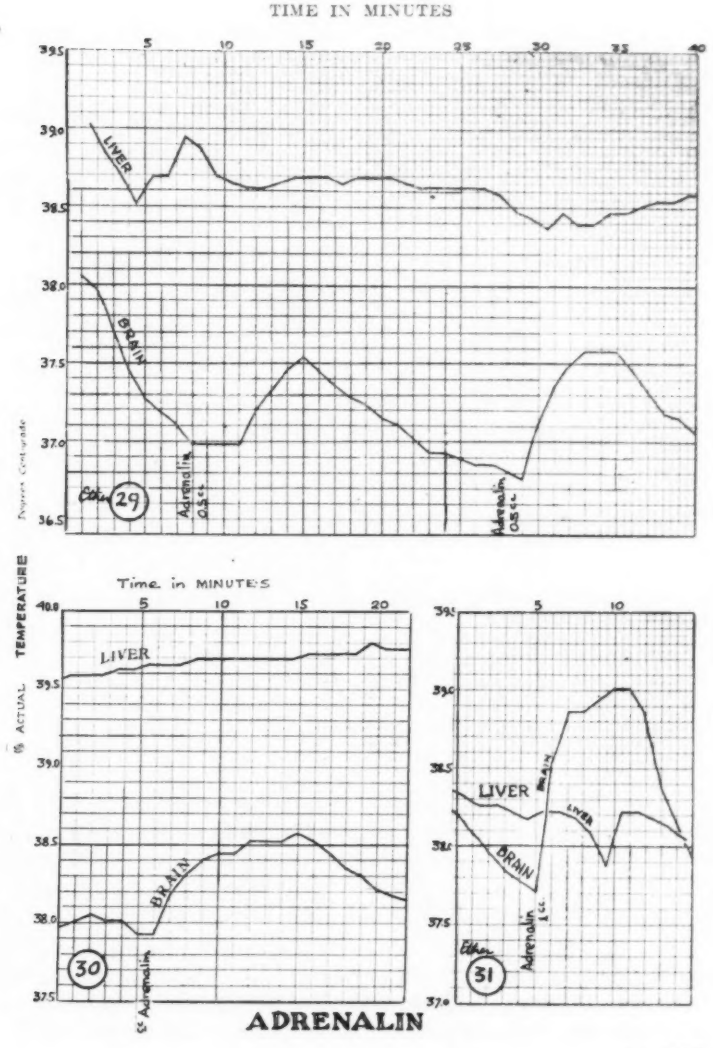
In section II of this presentation, we have reported a series of studies of the effects of various agents upon the temperature of the brain and the liver as indicated by measurements by means of especially devised thermocouples.

Early in the progress of these studies it became evident that the response of the brain to the injection of adrenalin was so uniformly manifested in normal animals by typical temperature changes that we adopted the temperature response of the brain to adrenalin as a kind of unit of comparison in a series of experiments, the findings in which appear to be so significant that we make them the subject of a separate report.

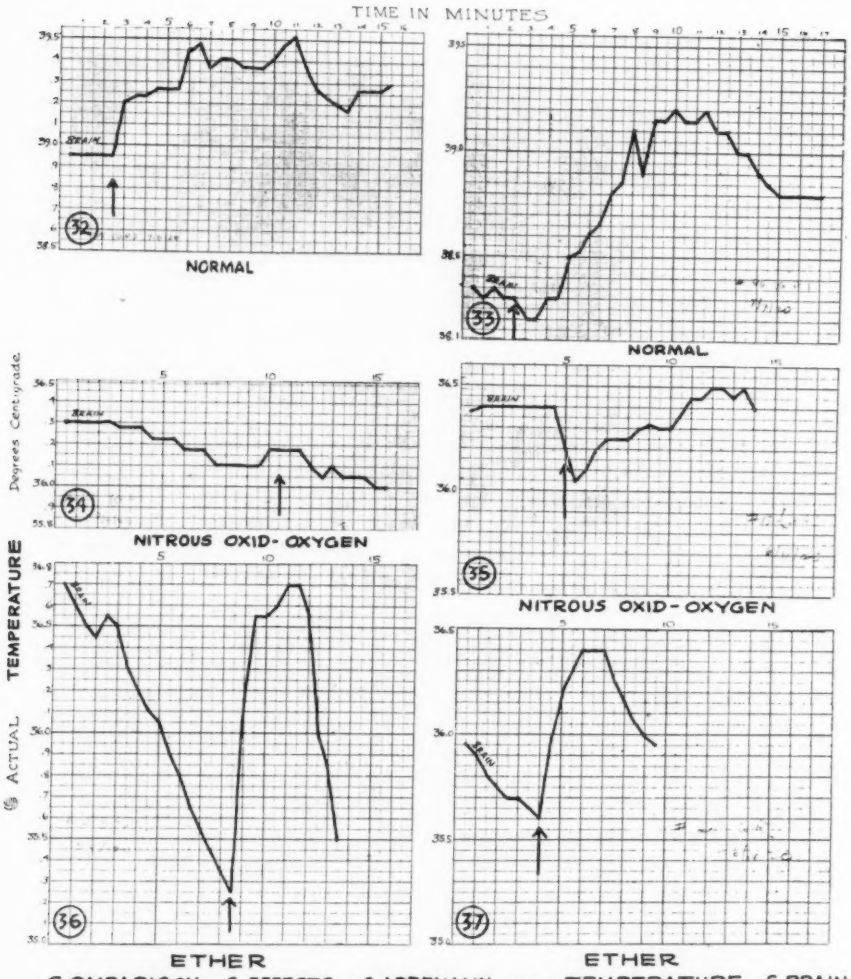
Four-tenths cubic centimeter per kgm. of 1:1000 Parke, Davis & Co.'s adrenalin was the dose employed throughout these experiments after a series of tests had shown that this dosage gave the most uniform response in normal rabbits.

Normal response of the brain to adrenalin. In normal rabbits the intravenous injection of adrenalin was followed by a rise in the temperature of the brain which in most instances amounted to between 0.3 and 0.5°C. (chart 30) although in some instances an even greater rise occurred (charts 32 and 33). The rise started immediately after the injection, the highest point being reached in from 6 to 10 minutes, with an approximately equally protracted return to the temperature level preceding the injection.

Response of the brain to adrenalin in anesthetized animals. In rabbits under ether anesthesia the temperature of the brain rose abruptly upon the injection of adrenalin, and the rise was usually greater than in normal animals (charts 29, 31, 36 and 37). Under *nitrous oxid anesthesia*, on the other hand, there was slight or no response to the



Charts 29 to 31. Comparison of the effect of adrenalin on the temperature of the brain in a normal animal (30) and in animals under ether anesthesia (29 and 31). Note the lack of response in the liver.



Charts 32 to 37. Comparison of the effects of adrenalin upon the temperature of the brain in normal animals and in animals under ether or nitrous oxid anesthesia.

injection of adrenalin or the response was atypical as in charts 34 and 35. (See also charts 36 and 37 in the preceding section.) In one experiment the injection of adrenalin in an animal anesthetized with urethane caused an abrupt rise in the temperature of the brain followed by a delayed decline (chart 38).

Response of the brain to adrenalin in iodized animals. The injection of adrenalin in animals which had been iodized by the intraperitoneal injection of iodoform produced an abrupt increase in the temperature of

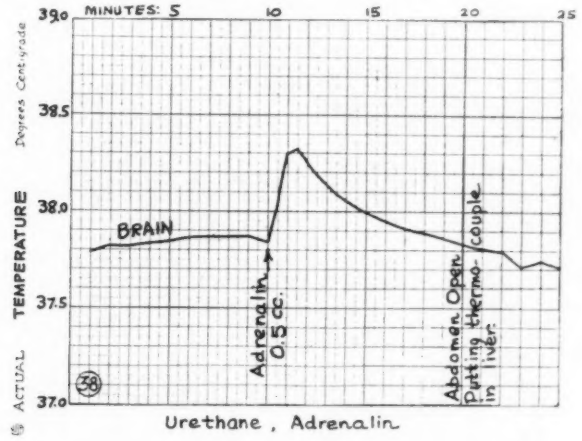
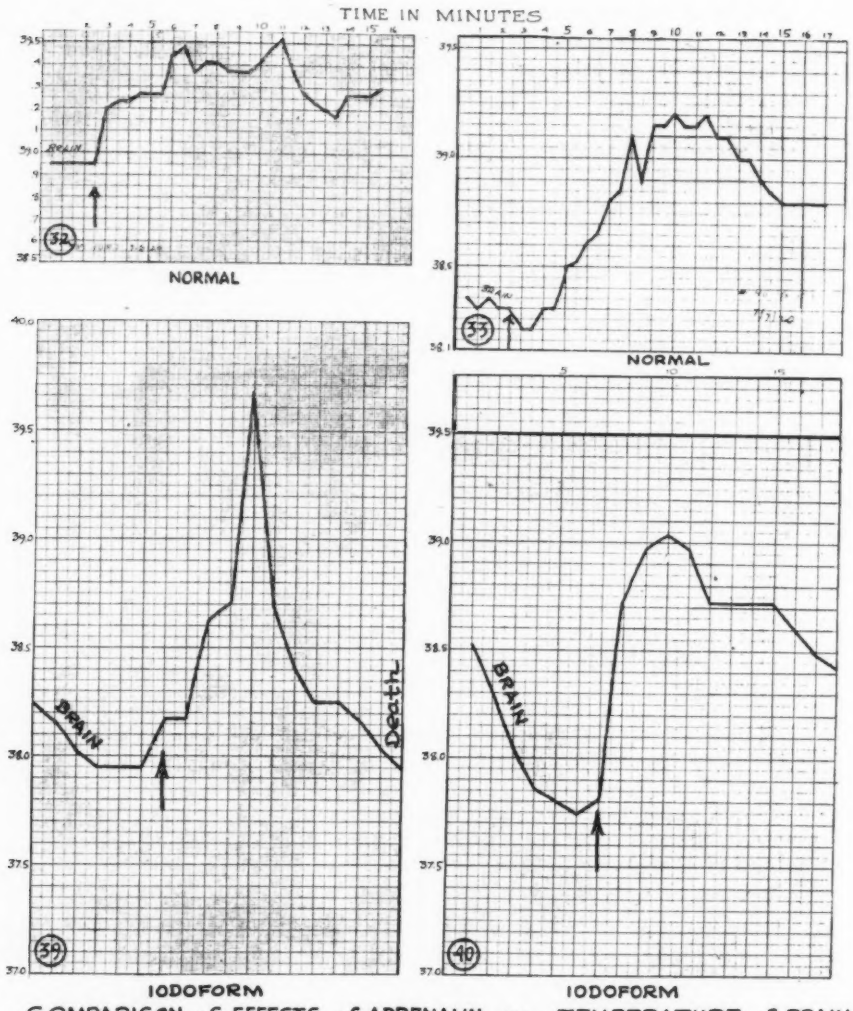


Chart 38. Effect of adrenalin upon the temperature of the brain in an animal anesthetized with urethane.

the brain which was greater than that observed in any other condition—in one instance amounting to 1.72°C. within 4 minutes with an equally abrupt fall (charts 39 and 40).

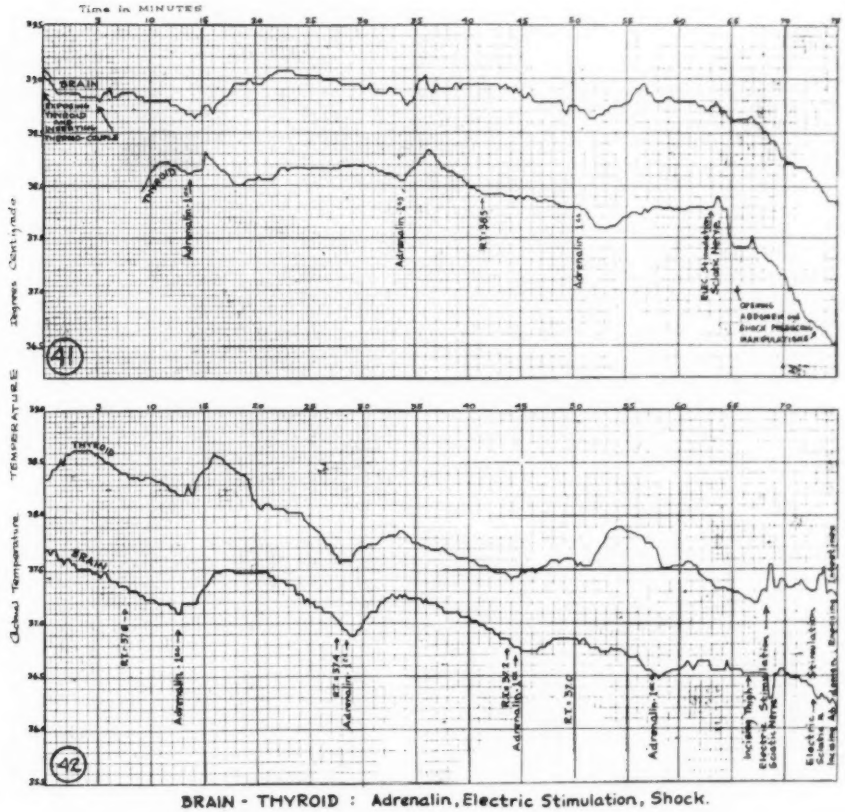
Response of other organs than the brain to the injection of adrenalin. The *liver* is apparently irresponsive to the injection of adrenalin as far as is indicated by temperature variations, as is shown by charts 29 to 31 and by the charts in the preceding report in which adrenalin injection was added to other forms of stimulation.

The *thyroid gland*, on the other hand, showed a response to adrenalin in temperature variations which in some cases approximately paralleled the temperature changes in the brain (chart 42).



Charts 39 and 40. Comparison of the effect of adrenalin on the temperature of the brain in normal and in iodized animals. The temperature curve is more abrupt in the iodized animals—as in hyperthyroidism.

The temperature of *voluntary muscle* apparently is not changed by adrenalin injection (charts 49 and 50).

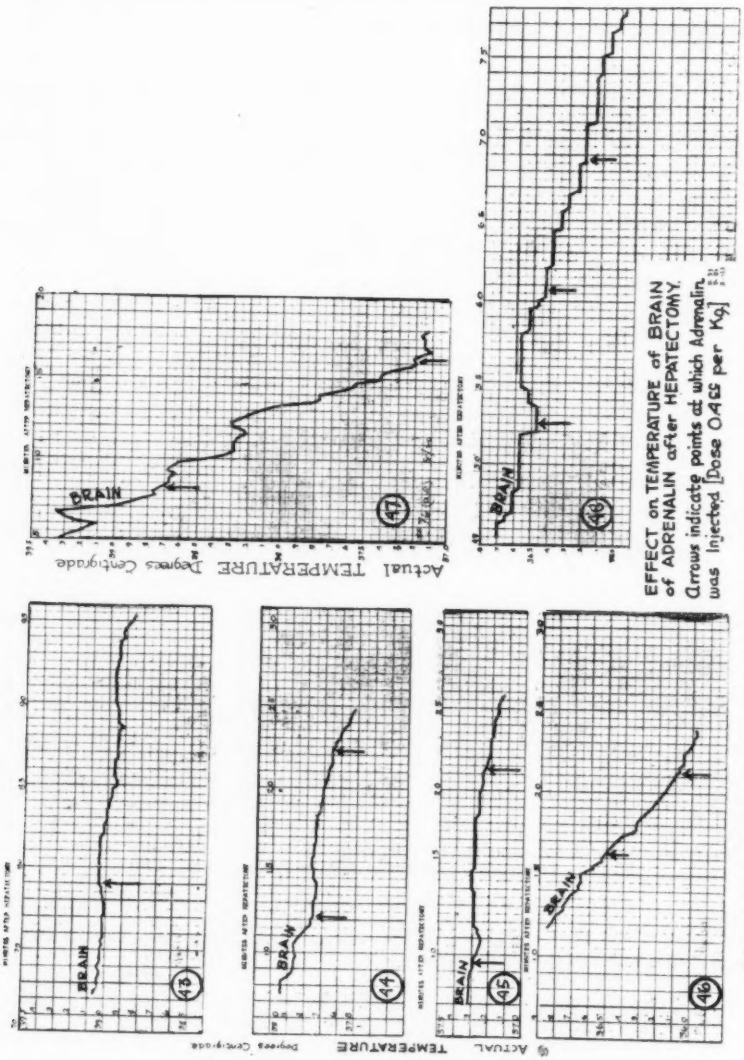


BRAIN - THYROID : Adrenalin, Electric Stimulation, Shock.

22-255

Charts 41 and 42. Comparison of the effects of adrenalin upon the temperature of the brain and the thyroid gland.

The effect of hepatectomy upon the response of the brain to adrenalin. Repeated observations appeared to demonstrate that after hepatectomy the power of the brain to respond to adrenalin is diminished or lost (charts 43 to 49).



Charts 43 to 48. Effect of adrenalin on the temperature of the brain in hepatectomized animals.

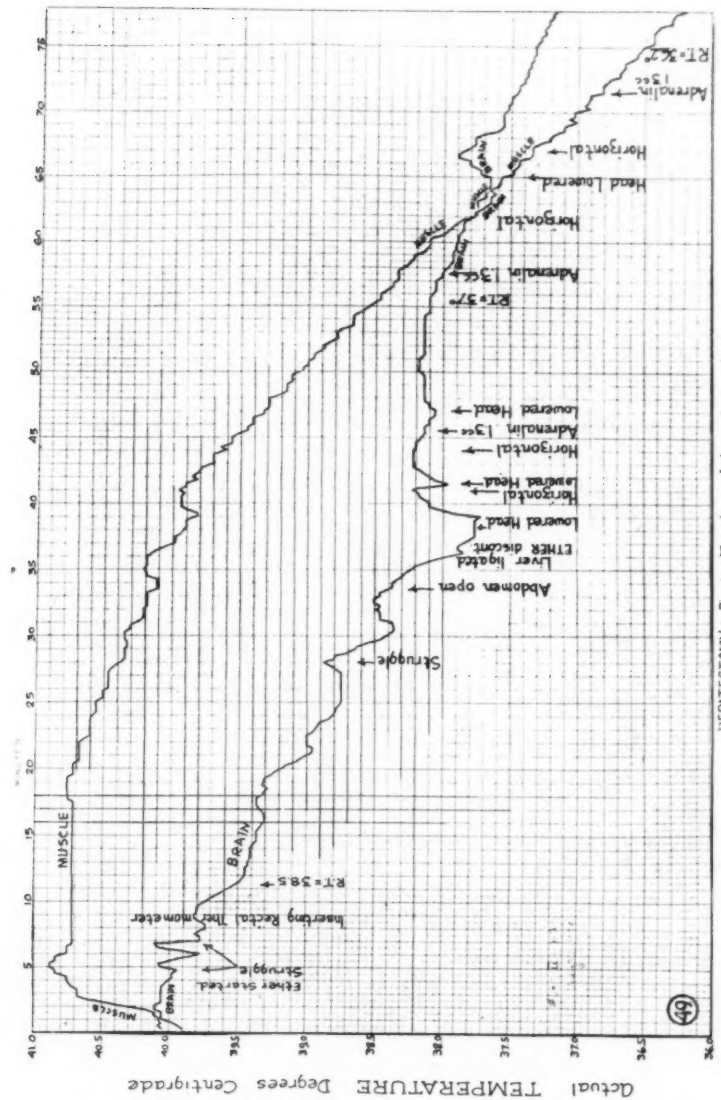


Chart 49. Effect of adrenalin upon the temperature of the brain in a hepatectomized animal. This chart shows strikingly the variations in temperature which may result from the manipulations essential to the ligation of the liver.

Note the lack of response of the muscle throughout.

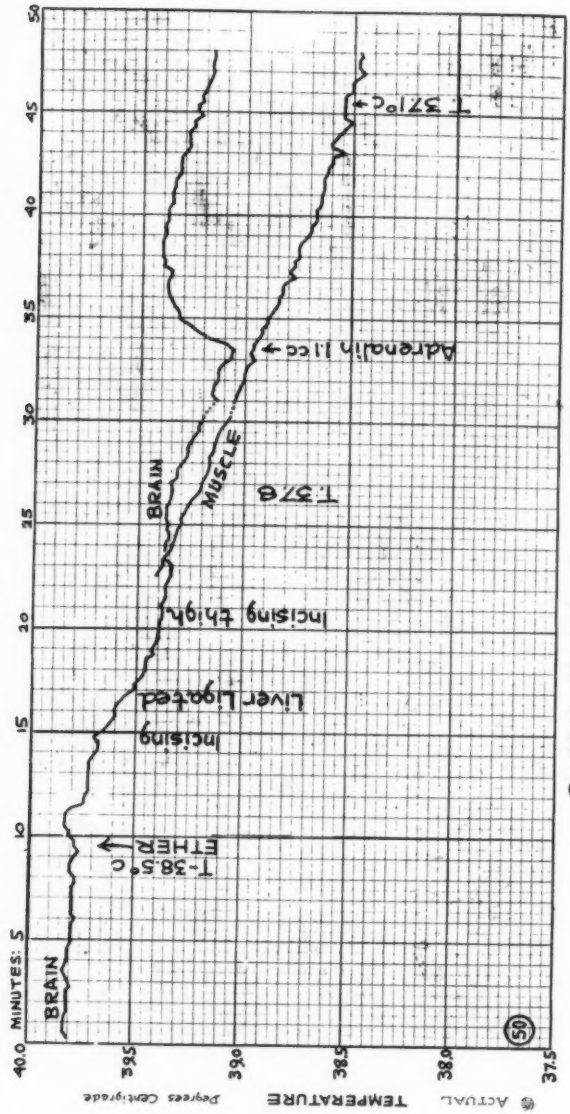


Chart 50. Effect of adrenalin on the temperature of the brain in an animal in which a small lobe of the liver was not included in the ligation. Note the lack of response in the muscle.

By accident, however, it was found that if even a very small portion of the liver remained patent to the circulation, the brain would register its usual response to adrenalin by the typical increase in temperature

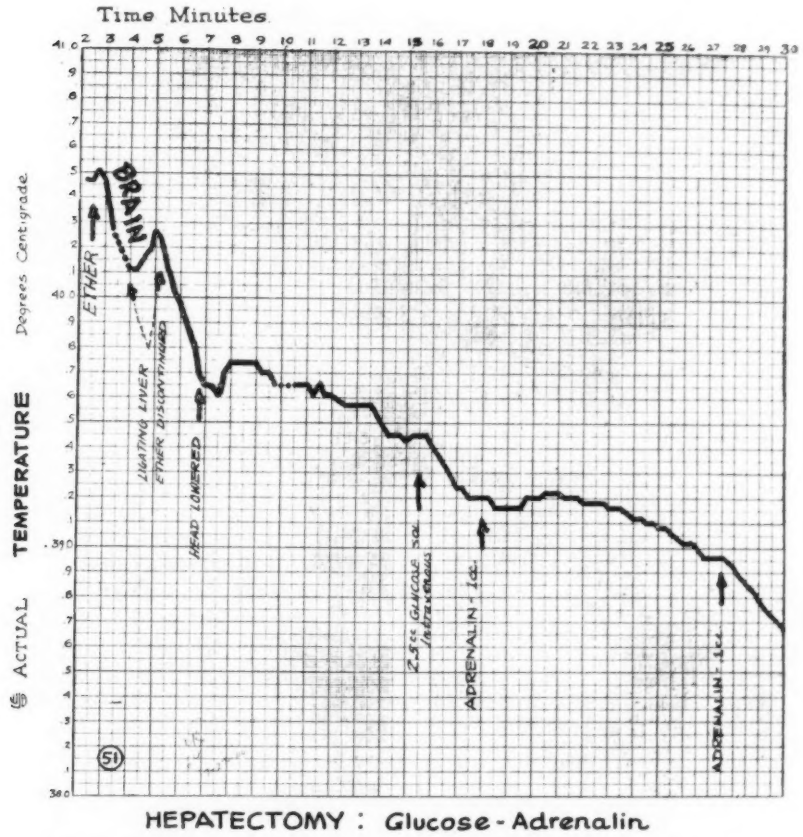


Chart 51. Effect of adrenalin upon the temperature of the brain in a hepatectomized animal which had received an intravenous injection of glucose.

(chart 50). In this experiment, it appeared at first that the premise we had thought established by preceding hepatectomies was disproved, until autopsy revealed that a small lobe of the liver had not been included in the ligation. Subsequent experiments have verified this finding.

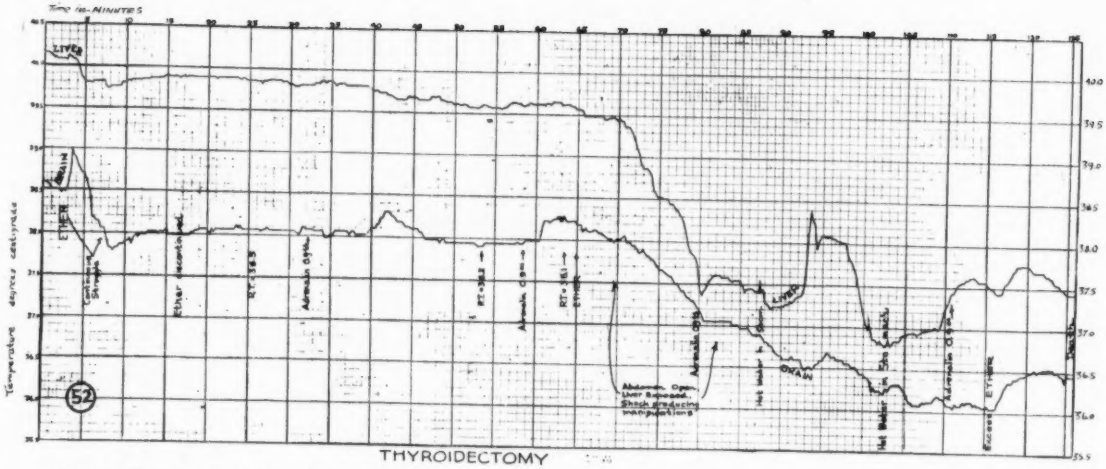


Chart 52. Effect of adrenalin; of abdominal trauma, and of hot water in the stomach; in an animal 48 hours after thyroidectomy.

Note the delayed and diminished response of the brain to adrenalin and the almost complete lack of response of the brain to the presence of hot water in the stomach.

A single experiment was performed to see whether or not lack of response of the brain of a hepatectomized animal was due to lack of glucose. In this experiment, at least, no effect upon the response to adrenalin was produced by the intravenous injection of a solution of glucose in a hepatectomized animal (chart 51).

The effect of thyroidectomy upon the response of the brain to adrenalin. Forty-eight hours after the removal of both thyroids the injection of adrenalin was followed by a delayed and slight rise in the temperature of the brain (chart 52).

SUMMARY

1. The temperature of the *brain* and of the *thyroid* was increased by adrenalin.

2. The temperature of the *liver* and of *voluntary muscle* was not affected by the injection of adrenalin.

3. In the *absence of the liver* the injection of adrenalin produced a diminished or no change in the temperature of the brain.

4. In the *absence of the thyroid* the reaction of the brain to adrenalin was diminished.

5. In *iodized* animals the reaction of the brain to adrenalin appeared more promptly and was greater than in normal animals.

6. In animals under *ether* anesthesia the reaction of the brain to adrenalin was greater than in normal animals.

7. In animals under *nitrous oxid* anesthesia the reaction of the brain to adrenalin was delayed and diminished.

GENERAL CONCLUSIONS

1. The variations in the temperature of animal tissues which accompany variations in function can be measured in the living animal by thermo-electric methods. In these studies the variations in temperature were measured to within 0.01°C.

2. Variations in temperature not only provide a further criterion whereby to identify the organs and tissues which are concerned in the production of vital phenomena, but also suggest the interrelation of the functions of these organs and tissues.

3. The variations in the temperature of the brain under various conditions parallel variations in the histologic picture and in the electric conductivity of the brain under the same conditions.

4. Of particular significance are the findings, *a*, that in voluntary muscular activity and as a result of the direct electric stimulation of a nerve the temperature of the brain and of the liver varies in opposite directions; *b*, that upon the introduction of hot water into the stomach the reaction of the brain in increased temperature precedes that of the liver; *c*, that the temperature of the liver is but little altered or is unchanged by the injection of adrenalin; of strychnin; of an acid; of an alkali.

5. The findings in these studies support the conclusions, *a*, that the brain is the tissue upon which depend the reactions of the organism to stimulation; *b*, that the thyroid and the adrenals play essential parts in the production and maintenance of these reactions; *c*, that in the performance of its function the brain is indissolubly linked with the liver.

6. The lack of response of the brain to adrenalin in the absence of the liver together with the opposite reactions of the brain and the liver may form vital links in the chain of evidence whereby we may determine the function of each in the electro-chemical operation of the animal mechanism.

VASODILATOR MECHANISMS

I. The Effect of Nicotine on the Depressor Reflex

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Contribution no. 95 from the Anatomical Laboratory of the Northwestern University Medical School

Received for publication July 10, 1922

Investigations of relatively recent date have clearly demonstrated the existence of vasodilator fibers. It is now known that the drop in blood pressure, caused by stimulation of the depressor nerve, or of the vagodepressor nerve when the former is included in the vagus, is due to two factors: an active vasodilatation, mediated through vasodilator fibers, and an inhibition of the tonic action of the vasoconstrictors. As an example of reflex excitation of vasodilator fibers, an experiment of Bayliss (1) may be cited. That investigator cut the cervical sympathetic trunk in the cat and thus eliminated all vasoconstrictor tone in the vessels supplying the submaxillary gland. He then stimulated the central end of the vagus and obtained an increased rate of blood flow through the gland. Since all vasoconstrictor fibers had been eliminated, the reflex vasodilatation could not be due to an inhibition of vasoconstrictor tone and must be ascribed to an active vasodilator mechanism. In the case of the submaxillary gland one might assume a reflex secretion and secondary action of metabolites on the blood vessels, but this explanation can not reasonably be applied in the other instances of reflex vasodilatation to be cited.

Tschalussow (2) by an ingenious method converted the nasal cavities into a plethysmograph and obtained vasodilatation in the nasal mucosa of the cat as a result of stimulation of the vagus nerve. Here again the vasoconstrictor fibers had previously been cut in the cervical sympathetic trunk and the results cannot be explained as a reflex inhibition of vasoconstrictor tone. Martin and Mendenhall (3) confirmed the findings of Tschalussow and also obtained evidence of an active vasodilatation in the nasal mucosa upon weak stimulation of the sciatic and saphenous nerves.

Tschalussow (4) and Fofanow (5) had both previously presented evidence to show that stimulation of the depressor nerve causes an active dilatation of the vessels of the entire body. In 1913 these two authors (6) published a series of experiments which they had carried out together. They determined the changes in volume of the tongue and the hind limb due to stimulation of the vagodepressor nerve. In these experiments the vasoconstrictor fibers for the tongue had been eliminated by section of the cervical sympathetic trunk and those for the hind limb by removal of the abdominal sympathetic chain. In this way a reflex inhibition of vasoconstrictor tone could be excluded; and yet upon stimulation of the central end of the vagus nerve the plethysmograph gave evidence of vasodilatation in both the tongue and the hind limb.

The work of Martin and Stiles (7), which will be discussed in another paragraph, also points to the conclusion that the drop in blood pressure from stimulation of the vagus nerve in the cat is in part due to an active vasodilatation.

The facts just presented clearly indicate the existence of vasodilator fibers, but with the exception of those in the chorda tympani and nervi erigentes great difficulty has been experienced in demonstrating their presence by direct stimulation of peripheral nerves. This difficulty arises from the fact that when a nerve containing both constrictor and dilator fibers is stimulated in the ordinary way the action of the constrictors is dominant. Vasodilatation of the leg has, however, been produced by mechanical and by slowly repeated electrical stimuli of the sciatic and by stimulating that nerve when it had been cooled.

Another difficulty has been the uncertainty as to the existence of any vasodilator fibers in the sympathetic trunk, to which one might naturally expect to trace all vasomotor fibers for the limbs. Bayliss (8), employing methods that have been successful in demonstrating vasodilator fibers in the sciatic, was unable to find any evidence of them in the abdominal sympathetic trunk. It is true that there are indications of the existence of such fibers in the cervical portion of the sympathetic trunk supplying the buccofacial region (9), (10) and the salivary gland (11). But these observations have been called in question by Bayliss (12) and Gaskell (13). Dale (14) has shown that ergotoxine reverses the effect of adrenin, causing it to produce a drop in blood pressure. This he interprets as a stimulation of sympathetic vasodilators after the constrictors have been paralyzed by ergotoxine. In the same way this toxin reverses the effect of stimulation of the splanchnic nerve

and makes it produce a drop instead of a rise in blood pressure. But the results of Dale might be interpreted as due to a reversal of the normal action of the vasoconstrictors and does not prove the existence of vasodilator fibers.

While all of the vasoconstrictor fibers pass through the sympathetic chain, most if not all of the autonomic vasodilators pass through either the cranial or the sacral outflows; and it remains an open question whether there are any vasodilator fibers in the thoracico-lumbar autonomic or "sympathetic" system. Perhaps the most important and widely distributed vasodilator fibers may prove to be those of the dorsal roots.

Bayliss (15) has shown on what appears to be satisfactory evidence that at least the majority of the vasodilator fibers for the hind limb have their cells of origin in the lower lumbar and first sacral spinal ganglia. Stimulation of the corresponding dorsal roots proximal to the ganglia, after the roots have been cut away from the spinal cord, causes vasodilatation of the leg; and this effect is not lost even after time has been allowed for the degeneration of any fibers having their cells of origin in the cord. If, however, the spinal ganglia are removed and time allowed for degeneration, stimulation of the distal part of the root no longer causes vasodilatation. He believes that these vasodilator fibers are identical with the sensory fibers and that the vasodilator impulses are conducted antidromically. Bayliss also believes that in reflex vasodilatation the impulses leave the spinal cord by way of the sensory fibers of the dorsal roots, although he recognizes the theoretical difficulty involved in explaining how the impulses are transmitted to the sensory fibers within the spinal cord. This obviously would represent a reversal of the law of the dynamic polarity of the neurons, which requires that an impulse shall cross a synapse only from axon to dendrite or cell body, never from axon to axon.

According to Bayliss' theory the vasodilator fibers, unlike the vasoconstrictor fibers, do not run through the sympathetic trunk and are not interrupted in sympathetic ganglia. A single fiber with trophic center in the spinal ganglion is supposed to carry the impulses from the spinal cord to the blood vessels. It will be obvious that the use of nicotine in doses sufficient to interrupt the passage of impulses through the sympathetic ganglia should throw light on the course taken by the vasodilator impulses from the spinal cord. If the mechanism corresponds in all points to the account of it given by Bayliss, nicotine should not prevent reflex vasodilatation resulting from central stimulation of

the vagus nerve. Abolition of this reflex by suitable doses of that toxin would indicate that the impulses passed through synapses of sympathetic character. This would require a modification of Bayliss' theory and make it necessary to search for the synapses in which the interruption occurred. For reasons which will be made apparent in the next paper of this series (p. 403), the presence or absence of such interruptions in the spinal ganglion cannot be determined by direct stimulation of the dorsal roots in a nicotinized animal; but the vasodilator reflex described by Martin and Stiles offers a means for investigating this question.

Martin and Stiles (7) have presented evidence which makes it extremely probable that the depressor reflex resulting from central stimulation of the cat's vagus consists of two parts; stimulation of a vasodilator and inhibition of a vasoconstrictor mechanism. Employing quantitative methods of measuring electrical stimulation they found that the vagus nerve of the cat has two thresholds. A stimulation of about 10Z units causes a moderate and transient fall in blood pressure. Increasing the strength of the stimulus beyond this point caused little if any increase in the extent of the drop until its intensity reached about 250Z units. Stimulation of this magnitude caused a much greater drop which could be maintained for a longer period of time. These differences are illustrated in figure 2, *I*, where *v15* represents a weak and *v7* a strong stimulus of the central end of the vagus. They would interpret the first response as due to the stimulation of the vasodilator mechanism and the second to the entrance of another and more powerful factor, the inhibition of vasoconstrictor tone.

The present investigation was undertaken to determine the effect of nicotine on these depressor reflexes. If the theory of Bayliss were entirely correct nicotine in moderate doses should not completely abolish this reflex; and, according to Martin and Stiles' conception, it should not affect the response to weak stimulation at all but would reduce that from strong stimulation.

Technique. Cats under ether, administered through a tracheal cannula, were prepared for carotid blood pressure tracings. Both vagi were cut low in the neck, their central ends secured by ligatures and carefully protected against drying, cooling, or contamination with citrate solution. The ulnar and median nerves were exposed, included in a single ligature and cut distally. In most experiments the vasomotor reflexes from stimulation of the vagus were first tested, then nicotine was administered intravenously and the reflexes tested during the period

of paralysis and again after recovery from the effects of the toxin. In others nicotine paralysis was induced first and the vagal vasomotor reflexes registered during the paralysis and in various stages of recovery. Experiments, in which for any reason we failed to get a vasodilator reflex either before the nicotine was administered or after the animal had recovered from the effect of the poison, were discarded.

Results. Tracings obtained from cat III will serve to illustrate the nature of the results obtained. A weak faradic stimulus applied to the central end of the vagus caused a moderate drop in blood pressure (fig. 1, *I*). At 9.58, 2.5 mgm. of nicotine were administered intravenously. This caused a marked rise in blood pressure followed by a drop

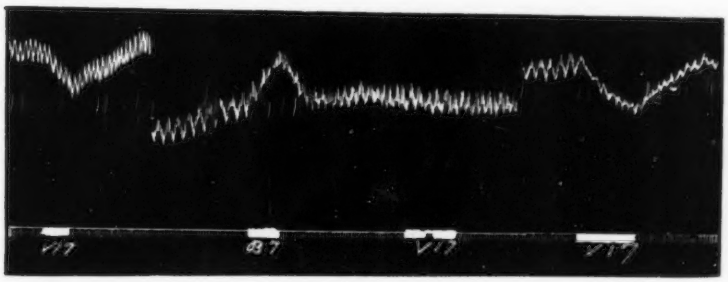

Fig. 1. Carotid blood pressure tracings from a cat. *I*, weak stimulation of the central end of the vagus, secondary coil at 17. *II*, 10 minutes after intravenous injection of 2.5 mgm. of nicotine; *B7*, strong stimulation of brachial nerves, secondary coil at 7; *v17*, weak stimulation of vagus, secondary coil at 17. *III*, 17 minutes after the injection of nicotine, weak stimulation of the vagus, secondary coil at 17.

of about 50 per cent below the original level. The pressure remained low for several minutes and then began to rise slowly. At 10:08, while the pressure was rising, a strong stimulus was applied to the central ends of the two brachial nerves and gave a typical pressor reaction (fig. 1, *II*, *B7*). A minute later the same weak stimulation of the vagus which had previously given a clear cut depressor reflex was without effect (fig. 1, *II*, *v17*). At 10:15 the blood pressure had returned nearly to the original level and weak stimulation of the vagus again gave a marked depressor reflex (fig. 1, *III*). Since the weak stimulation used in this experiment was obtained with the secondary coil at 17 and was very near the vagus threshold, we may conclude, according to Martin

and Stiles, that the drop in blood pressure was due to active vasodilatation. According to this interpretation the vasodilator mechanism was more susceptible to nicotine in this cat than the vasoconstrictor mechanism, since the pressor response was the first to return during the period of recovery.

It was possible, by giving repeated small doses of nicotine and allowing sufficient time between for recovery, to cause the depressor reflex from weak stimulation to disappear and reappear several times in the course of one experiment. This is illustrated in figure 2 and the accompanying protocol.

Langley and Dickinson (10) studied the "progressive paralysis of different classes of nerve cells in the superior cervical ganglion." They administered nicotine intravenously, either in successive small doses and noted the order in which paralysis appeared, or in one large dose and then watched the order of recovery. A dose of 5 or 10 mgm. paralyzed all of the cells of the superior cervical ganglion of the rabbit. They found the order of recovery to be inversely as the order of paralysis. The maximum effect developed within 5 minutes after the injection and full recovery occurred within 30 minutes.

In dosage of nicotine required and in the time relations of paralysis and recovery our results on the obliteration of the vasodilator reflex are in such close accord with those of Langley and Dickinson that it is difficult to avoid the conclusion that that reflex has been obliterated as a result of the action of the nicotine on sympathetic synapses located somewhere in the efferent vasodilator path. It is true that while nicotine has a selective affinity for the sympathetic ganglia large doses will depress somatic reflexes and stop respiration. That the action in question is not of this character is evidenced by the fact that the vasodilator reflex is in some cases, as in the experiment just cited, eliminated by very small doses of nicotine. In lightly anesthetized animals, when nicotine has eliminated the vasodilator reflex, it is still possible to get struggling and changes in respiratory rhythm from vagus stimulation. The vasodilator reflex disappears and reappears at about the same time as known sympathetic responses but may be absent when vasoconstriction and dilatation of the pupil are still obtainable.

Our series of experiments is not extensive enough to enable us to say whether the vasoconstrictor or vasodilator reflex is the more susceptible to nicotine. In the cat whose record is reproduced in figure 1 the vasodilator reflex was the more susceptible while in another whose record is reproduced in figure 2 the vasoconstrictor mechanism was more easily eliminated.

Another point which we have not had time to investigate thoroughly is the difference in the effect of nicotine on reflex vasoconstriction and reflex inhibition of vasoconstrictor tone. One would expect that paraly-

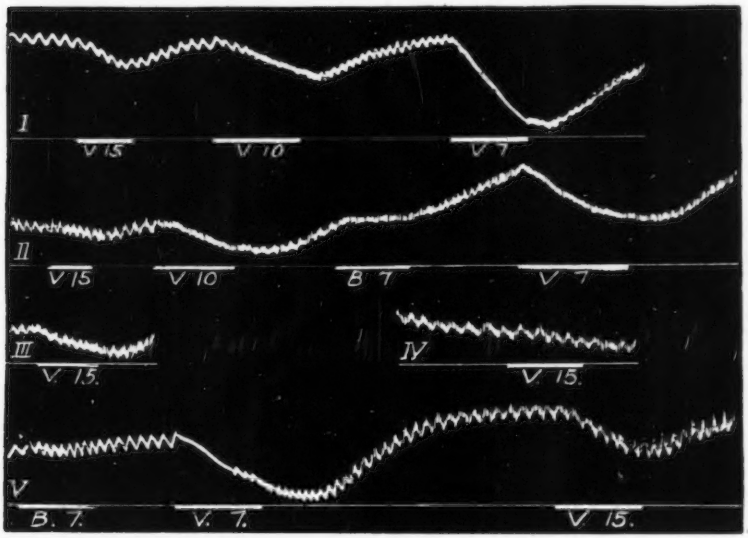

Fig. 2. Carotid blood pressure tracings from a cat. Electrical stimulation of the central end of the vagus, *V*, and of the brachial nerves, *B*. Arabic numerals after *V* and *B* indicate the position of the secondary coil. *I*, Before injection of nicotine, base line raised 48 mm., shows the depressor reflex from weak, moderate and strong stimulation of the vagus nerve. *II*, base line raised 35 mm., shows vascular reflexes after 2.5 mgm. of nicotine had caused a moderate drop in blood pressure. Weak stimulation of the vagus, *v15*, at the end of the drop caused by nicotine was practically without effect. Moderate vagus stimulation, *v10*, at the beginning of the recovery-rise gave a drop nearly as great as before, although strong stimulation of the brachial nerves, *B7*, did not produce a vasoconstriction. A little later and while the blood pressure was still recovering from the effects of the nicotine strong vagus stimulation, *v7*, caused a marked drop in blood pressure. *III*, base line raised 63 mm., after full recovery from the first dose of nicotine, reappearance of the depressor reflex from weak stimulation of the vagus, *v15*. *IV*, base line raised 38 mm., at the end of a period of falling blood pressure caused by a second dose of 2.5 mgm. of nicotine, no response from weak stimulation of the vagus, *v15*. *V*, base line raised 44 mm., late in the period of recovery from the second dose of nicotine, vasoconstrictor response from strong stimulation of the brachial nerves, *B7*, was absent, though strong stimulation, *v7*, and weak stimulation of the vagus, *v15*, gave the normal responses.

sis of the synapses in the vasoconstrictor path would eliminate both reflexes at the same time. But this was not usually the case. In some partially nicotinized animals strong stimulation of the vagus would give the marked drop in blood pressure indicative of reflex inhibition of vasoconstrictor tone at a time when a pressor response could not be obtained from the brachial nerves. In others the pressor reflex could be elicited from the brachial nerves when no vasomotor response could be obtained from the vagus.

The moderate drop in blood pressure resulting from weak stimulation of the vagus seems to be the response that is most easily eliminated but without giving excessive doses every trace of a depressor reflex can be made to disappear. It is true that nicotine lowers the level of the blood pressure but not to a point where this becomes a serious factor. In tracing *II* of figure 2 the initial blood pressure was 84 and in tracing *IV* it was 86 (see protocol). This is somewhat higher than in figure 1, *I*, which shows a clear-cut depressor reflex.

Of course it would be desirable to check these results by plethysmographic records of the volume of the leg in cats in which the abdominal sympathetic trunk had been removed and in which all vasoconstrictor tone in the vessels of the leg had thus been eliminated. But, since a fall in blood pressure tends to neutralize the effect of vasodilatation so far as the volume of the leg is concerned, there would be great difficulty in applying this test.

Our experiments show that the drop in blood pressure from weak stimulation of the vagus, which, following the interpretation of Martin and Stiles we have interpreted as evidence of active vasodilatation, is readily eliminated by nicotine. Furthermore, every trace of a depressor reflex from even strong stimulation of the vagus can be eliminated without giving an excessively large dose. This demonstrates that if the vasodilator fibers of the dorsal roots participate as efferent fibers in this depressor reflex the impulses which they carry pass through a synapse of sympathetic character.

In the second paper of this series we shall show that no clear evidence has ever been presented that the sensory fibers of the dorsal roots actually convey vasodilator impulses from the spinal cord. Our results require a modification of Bayliss' theory of antidromic conduction in such a way as to allow for a synaptic interruption in the vasodilator path.

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VASODILATOR MECHANISMS

II. The Vasodilator Fibers of the Dorsal Roots

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Contribution no. 96 from the Anatomical Laboratory of the Northwestern University Medical School

Received for publication July 10, 1922

In 1876 Stricker (1) reported the existence of vasodilator fibers for the hind limb of the dog in the dorsal roots of the lower lumbar nerves. After these roots had been divided, mechanical or electrical stimulation of their peripheral ends caused a rise of temperature in the skin of the leg, a result which Stricker interpreted as evidence of vasodilatation. He mistakenly designated the lowest lumbar nerve as L. V. instead of L.VII. The same year Crossy (2) repeated Stricker's experiments, using the L.IV and L.V dorsal roots, with negative results. Stricker (3) then conducted a second series of experiments and obtained elevations of as much as 10° in the temperature of the skin. His results were confirmed for the hind leg and similar observations made on the fore limb by Bonuzzi (4). Gaertner (5) ligated the L. VI and VII dorsal roots intradurally and separated them from the spinal cord. This caused a rise in temperature of the skin; and after allowing time for the temperature to fall it would be raised again by mechanical or electrical stimulation of distal ends of the cut roots. Evidence of vasodilator fibers in the dorsal roots was also obtained by Morat (6) and by Hasterlink and Biedl (7). Morat noted a reddening of the skin when the dorsal roots were stimulated.

Werziloff (8) was the first to investigate this question with modern methods. He was able to confirm the results of previous investigators both by observations of the color of the skin and by the use of the thermometer. But he also used the plethysmograph and studied the blood pressure and rate of flow in the veins of the limb. Contrary to the findings of Gaertner, Werziloff found that section of the dorsal roots of the lower lumbar and first sacral nerves caused a drop in the temperature of the skin, just as if some tonic action of the vasodilators had been

cut off. But it is doubful if the dorsal root fibers do exert such a tonic dilator effect on the blood vessels since this observation of Werziloff is out of harmony with the results of other investigators. In agreement with other workers, Werziloff found that the L. VI and VII and S. I dorsal roots contained the greater part of the vasodilator fibers for the hind limb, though there were also some in the L. IV and V dorsal roots. Mechanical and electrical stimuli of the distal ends of the five roots enumerated caused a rise in the temperature of the skin. Section and stimulation of the ventral roots did not have this effect.

With the plethysmograph Werziloff registered an increase in the volume of the leg and an increased amplitude of the pulse waves when the dorsal roots were stimulated. In order to check these results he put a cannula into the saphenous artery and another in the small saphenous vein. Stimulation of the dorsal roots caused a fall in arterial and a rise in venous pressure. At the same time the respiratory variations in venous pressure disappeared and oscillations synchronous with the pulse appeared. The rate of flow from the saphenous vein was increased and the escaping blood became redder when the dorsal roots were being stimulated.

Because they were contrary to the Bell-Magendie law the observations of Werziloff and earlier workers were received with skepticism until after the publication of Bayliss' (9) convincing paper in 1901. His plethysmograph tracings give indisputable evidence of vasodilatation from stimulation of the L. V, VI, VII and S. I dorsal roots. The response occurred chiefly in the cutaneous blood vessels. He obtained the same results after excision of the abdominal sympathetic trunk, thus demonstrating that the vasodilator fibers of the dorsal roots do not pass through that trunk. Section of the dorsal roots proximal to the spinal cord with time allowed for the degeneration of any fibers having their trophic center in the spinal cord did not affect the results. But the vasodilator fibers degenerated when the spinal ganglia were extirpated. Nicotine applied directly to the spinal ganglia did not interrupt the passage of vasodilator impulses through these ganglia. These experiments show that the vasodilator fibers of the dorsal root have their cells of origin in the spinal ganglion and yet run without interruption through the ganglion into the mixed nerve; and in these respects they correspond exactly to ordinary sensory fibers. Bayliss concluded that they were identical with the sensory fibers to the blood vessels. According to thi theory the fibers in question must conduct impulses in both directions; and to express this idea Bayliss applied

the adjective antidromic to the vasodilator impulses. Similar fibers for the fore limb were found in the C. VI, VII, VIII and T. I dorsal roots. No evidence of vasodilatation could be obtained by stimulating the ventral roots.

In a later paper Bayliss (10) presented inconclusive evidence that in at least some cases of reflex vascular dilatation of the limbs there is a production of antidromic nerve impulses in the dorsal root fibers. Similar experiments are reported by Fofanow and Tschalussow (11) but here again the evidence is far from convincing. It is at this point that the theory of antidromic conduction comes into conflict with our notions of the organization of the nervous system. The idea requires that the dilator impulses pass in the spinal cord from the terminal ramifications of one set of axons to the branches of another set of axons. Now this is a type of synapse which is unknown and we are certainly not justified in assuming its existence unless the observed phenomena can be explained in no other way.

It was to secure additional information on this phase of the problem, i.e., whether any vasodilator impulses do actually pass from the spinal cord to the blood vessels of the limbs and if so along what fibers they leave the cord, that the present series of investigations was begun. But in view of the skepticism which still exists concerning the phenomena of antidromic conduction it was necessary first of all to confirm the existence of vasodilator fibers in the dorsal roots.

Technique. Dogs of varying size and age were used for these experiments. Anesthesia was induced with ether, morphine (60 to 100 mgm. according to body weight) injected subcutaneously, tracheotomy performed and ether bottle attached, both vagi cut, laminectomy done and dura opened, cord ligated at level of L. VI segment, and the right L. VI and VII and S. I dorsal roots ligated separately and cut close to the cord. Care was taken to prevent the loss of body heat. In other dogs after the cord had been ligated a thread was passed under each of these three dorsal roots and the corresponding ventral roots were severed. In these cases the dorsal roots were ligated and cut after the plethysmograph had been applied.

The usual plethysmographic technique involves the making of an air-tight joint between the rubber cuff and the part of the leg projecting into the instrument. Bayliss and other investigators have accomplished this by having cuffs of various sizes to fit different sized legs and by spreading vaseline over the leg where it was in contact with the cuff. It is very difficult to make an air-tight joint without produc-

ing constriction. Any movement of the animal disturbs the adjustment and requires resetting of the instrument. To obviate these difficulties an air-tight rubber stocking was made of the same shape and size as the plethysmograph. The open end of the former was drawn over the open end of the latter and securely tied in place. A little beeswax in the groove around the open end of the instrument helps to make the joint air-tight. Before applying the plethysmograph both it and the attached stocking are filled with air. The animal's foot and leg are then made to invaginate the small end of the stocking until the leg covered by the invaginated stocking is within the plethysmograph. The instrument should be of such a size that, when the foot rests against the inside of the small end, the large end is at about the middle of the thigh and does not produce any constriction. When supported by clamps and an iron stand the plethysmograph should hold the leg rigidly and should be slanted outward and backward. If it is directed vertically upward pressure is likely to be exerted on the femoral artery. Warm water (40°C.) is then introduced through a tube opening into the side while the air escapes through another tube attached to the small end of the instrument. Since the leg is directed obliquely upward the weight of the water is sufficient to hold the rubber dam in firm contact with the leg and to keep it stretched tight at the open end of the plethysmograph. A little air is left in the upper end of the instrument and the tambour and connecting tube contain air. The membrane of the tambour should not be stretched too tight and the connecting tube should be as short as possible. The rubber stocking has the advantage over the cuff and vaseline in that it can be more quickly applied and requires no particular skill or experience for its use. In fact, with this method it is fully as easy to take a tracing of the volume of the leg as one of the blood pressure. The full equipment as used in these experiments can be obtained from Max Wocher & Son, Cincinnati, O. Figure 2, p. 399 shows what can be accomplished in this way in recording pulsations and volume changes in the leg.

The dorsal roots were stimulated with a faradic current of moderate strength. In many of the experiments the L. VII dorsal root was followed through the dura and separated from the corresponding ventral root to a point beyond the spinal ganglion. In these cases the effect of stimulation of the dorsal root could be compared with that of stimulation of the spinal ganglion.

Mechanical stimulation usually proved more effective than electrical. This was exerted by crushing the root or ganglion bit by bit

with an artery forceps starting proximally and making successive bites more and more distally. This mechanical stimulation however could in the nature of things be used only at the end of the experiment.

When not being stimulated the roots were protected by a pad of cotton wet with warm salt solution.

Results. The results of our experiments confirm the conclusions reached by Bayliss and earlier workers that the dorsal root contains vasodilator fibers. After the lower lumbar and first sacral dorsal roots have been divided close to the spinal cord, a moderately strong faradic stimulus applied to one of them just distal to the cut causes a dilatation of the blood vessels of the hind limb. In this situation the roots are long and can be isolated for a distance of a couple of inches which is much more than sufficient to prevent an escape of current to the ventral roots. The best results were obtained from the L. VII and S. I dorsal roots.

Mechanical stimulation is even more effective than electrical. The greatest dilatation has been obtained by crushing the root with an artery forceps beginning proximally and destroying the root a little at a time. In figure 1 the increased volume of the leg registered by the plethysmograph was due to the vasodilatation caused by crushing the L. VII dorsal root. Ligation of the roots is also an effective stimulus.

In some of the dogs after ligating the cord at the sixth lumbar segment we cut the ventral roots of the lower lumbar and sacral nerves, and slipped a thread under the dorsal roots. Then after the plethysmograph was applied the L. VI and VII and S. I dorsal roots were ligated in quick succession. Under these circumstances the plethysmograph recorded an increase in the volume of the leg. In one instance the small (or posterior) saphenous vein was opened and a record made of the rate of venous flow, each drop being recorded separately by a signal marker. When the L. VI and VII and S. I. dorsal roots were ligated in quick succession there was a marked increase in the rate of flow from this vein.

The tracings which we have reproduced were selected from our records of vasodilatation following mechanical stimulation because with this form of stimulus there is no possibility of concomitant excitation of neighboring structure such as the ventral roots or spinal cord. Although there still exists a great deal of skepticism concerning the existence of vasodilator fibers in the dorsal roots any one who will take the pains to acquire the requisite skill in the necessarily difficult manipulations involved can readily convince himself of the existence of such fibers.

Bayliss mentions an increase in the amplitude of the pulse wave in the plethysmograph tracing as of equal value with the rise as indicating vasodilatation. This is certainly true in the vasodilatation resulting from muscular contraction and in that caused by nitroglycerine, acetylcoholine and other dilator drugs (see fig. 4, p. 414). But in our experiments we never obtained an increase in the amplitude of the pulse from stimulation of the dorsal roots although in many instances we obtained a considerable rise indicating a rather large vasodilatation (figs. 1 and 2). This difference in the plethysmographic records of the effect of drugs and of stimulation of the dorsal roots may be due to a greater vasodilatation obtained with the former. However, we think it quite possible that the dorsal root vasodilators affect chiefly the capillaries and smaller arterioles. If this were the case a very considerable increase in the volume of the limb might occur without any increase in the amplitude of the pulse waves. Dr. D. R. Hooker tells us that in his observation on capillary circulation he has never seen a pulse in the arterioles or capillaries although he believes that under certain conditions a pulse may be transmitted through the capillaries to the veins. The observations of Krogh, summarized in a following paragraph, tend to show that at least a part of the dilator fibers of the dorsal roots go to the capillaries. Largely due to the work of the two investigators just mentioned, we know that the capillaries play an important part in vasomotor responses. The fact that in our experiments no increase in the amplitude of the pulse could be observed at least suggests the possibility that the response was largely in the capillaries.

For some reason better reactions are usually obtained from stimulating the spinal ganglion than from stimulating the dorsal root proximal to the ganglion. In making these tests it is of course necessary to separate the ventral from the dorsal root to a point beyond the ganglion and to carefully guard the ventral root from stimulation. An escape of current to the ventral root cannot always be avoided when the electrodes are applied to the ganglion but whenever such an escape occurs it can easily be recognized by the sharp movement of the recording lever caused by muscular contraction. By applying the electrodes first to the root, then after an interval to the ganglion, and then later to the root again we have nearly always found the response from the ganglion to be considerably greater. The same results can be obtained by crushing first the root and then the ganglion, and this procedure, while it has the disadvantage that it necessarily terminates the experiment, has the great advantage that it is not open to the objection of a possible escape of current (figs. 1 and 2).

There is nothing in these results inconsistent with Bayliss' theory that the dilator fibers are identical with the ordinary sensory elements, for it may well be that the cell bodies of the sensory neurons are more susceptible to electrical and mechanical stimuli than the fibers. On the other hand, if it should later be shown that the spinal ganglia contain synapses between pre- and post-ganglionic vasodilator neurons the greater reactions obtained from the ganglia might be interpreted as due to the greater susceptibility of the nerve endings in the synapse or of the post-ganglionic fibers.

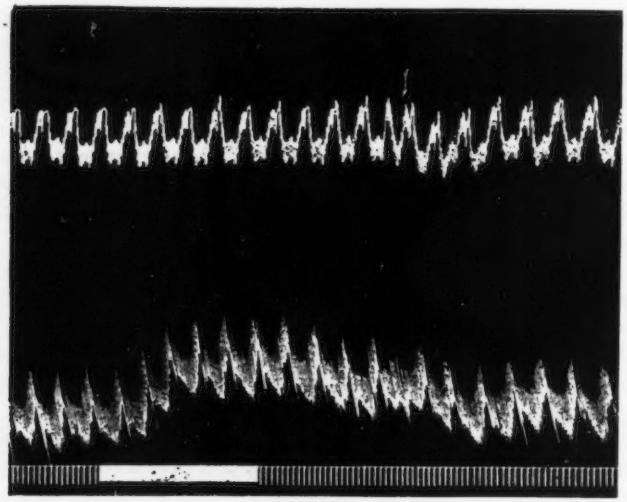

Fig. 1. Plethysmographic tracing of the leg below the carotid blood pressure tracing from a dog, showing the vasodilatation caused by crushing the L. VII dorsal root proximal to the spinal ganglion. The dog had previously been poisoned with nicotine.

Discussion. Taking into consideration all the facts in the case, it is hard to escape the conclusion that Bayliss is correct in identifying the vasodilator with the sensory fibers. That author showed that these fibers do not degenerate distally when the dorsal root is cut near the spinal cord, and hence they do not have their cells of origin in the cord. The cells are located in the spinal ganglia since the fibers degenerate when the ganglia are removed. Nicotine painted on these ganglia does not eliminate the vasodilator action of the dorsal roots which shows

that the impulses do not pass a synapse in the ganglion. The structure of the spinal ganglion has been studied in great detail by Dogiel (12), Cajal (13) and later by Ranson (14) and it is unlikely that any important elements entering into its composition have escaped observation. But the sensory neurons, each with its single process dividing into one branch directed centrally in the dorsal root and another directed distally in the nerve, are the only elements known which have their cells of origin in the ganglia and which are capable of conducting impulses through the ganglia without interruption. Bayliss believed

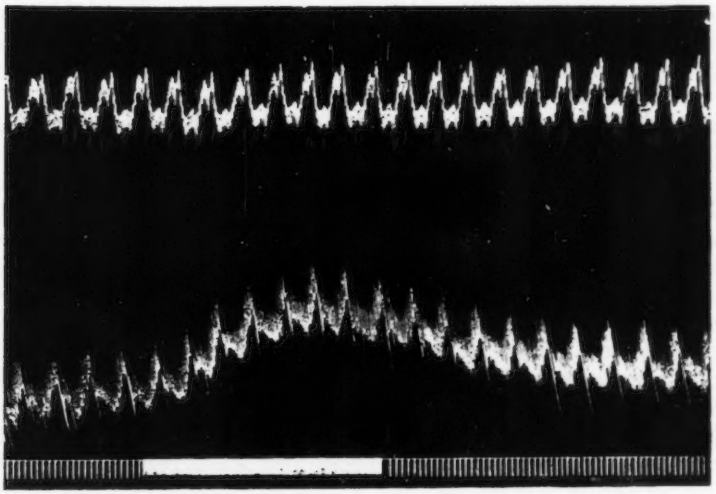

Fig. 2. Plethysmographic tracing of the leg below the carotid blood pressure tracing from a dog, showing the vasodilatation caused by crushing the L. VII spinal ganglion. This tracing was taken from the same nicotinized dog as that in figure 1 and only a few minutes elapsed between the two records.

that the sensory fibers to the blood vessels were capable of carrying impulses in the reverse direction and this he designated as antidromic conduction.

Strong support is given to this interpretation by the evidence of an axon vasodilator reflex furnished by Bruce (15), Januschka (16), Bardy, (17), Richardson and Wyatt (18) and Krogh (19). The inflammation caused by a drop of mustard oil is entirely prevented if the sensory nerves to the part are paralyzed by cocaine. Bruce showed that the

inflammation was due to irritation of the sensory nerve endings but that it was not eliminated by the section of the nerve unless time was allowed for the sensory fibers to degenerate. He found that the dilatation of local blood vessels in inflammation is not affected by the total section of the spinal cord, nor of the dorsal roots proximal to the spinal ganglia nor of the same distal to the ganglion unless time is allowed for degeneration. But if the sensory fibers are degenerated or if the part is under an anesthetic the vasodilatation does not occur. These facts he explains by the assumption that the sensory fibers give off side branches to the blood vessels and that inflammatory dilatation is produced by impulses carried up the sensory fiber to the side branch and then along this to the blood vessel. It is an axon reflex rather than a true reflex. The facts in the case have been confirmed by Januschka, Bardy and Richardson and Wyatt and the explanation falls directly in line with the vasodilator action of the sensory fibers as conceived by Bayliss.

Observations which can best be interpreted in terms of an axon vasodilator reflex have also been made by Krogh (19), who observed the reactions of the capillaries in the tongue of the frog under the microscope. Local irritation causes both the capillaries and arterioles to open. The reactions to local stimuli have nothing to do with true reflexes since they are in no way altered by the simple section or blocking of the nerves to the tongue. But the vasodilatation spreads over a larger area than that directly affected by the stimulus and this spreading is abolished by the action of cocaine and by the degeneration of the nerves to the tongue. These facts indicate that the phenomenon is in the nature of an axon reflex. Krogh believes that a nerve fiber accompanying an artery divides and innervates both the capillaries and intercapillary spaces. It must also give off branches to the artery since a stimulus of sufficient strength will cause the artery supplying the stimulated area to dilate even at a considerable distance.

Bardy (17) confirmed the work of Bruce but added the observation that the vasodilator axon reflex is eliminated by suitable doses of nicotine used intravenously or locally. He thinks that the branches of the sensory fibers going to the blood vessels are interrupted by sympathetic ganglion cells situated in the neighborhood of the vessels. In another place we have summarized what is known of the nerve cells in the plexuses accompanying the blood vessels (see p. 410). While such nerve cells as are required by Bardy's hypothesis have never been demonstrated, the assumption is in itself not unreasonable. But

if the hypothesis were correct nicotine in suitable doses should obliterate the vasodilator action of the dorsal roots. This we have found not to be the case. Nicotine can be injected into the blood stream in amounts sufficient to completely paralyze the vasoconstrictor neurons supplying the leg without decreasing the vasodilator action of the dorsal roots supplying that leg. In fact, the tracings reproduced in figures 1 and 2 were taken from a dog in which the vasoconstrictor impulses to the leg incased in the plethysmograph had been eliminated by nicotine. This accounts for the dilated arteries indicated by the great amplitude of the pulse waves in the plethysmographic tracing. The pulse waves are as high as when all the nerves to leg have been divided (see fig. 4, p. 414). The technique employed in injecting the nicotine is given in the following paper. Since the nicotine reached the leg in rather concentrated form, our experiments show conclusively that the branches from the sensory fibers to the blood vessels, whose existence we must assume to explain both the facts of antidromic conduction and axon-reflex vasodilatation, are not interrupted, as Bardy thought, by sympathetic ganglion cells.

The most unreasonable part of the theory of antidromic conduction is, as Bayliss realized, the assumption that within the spinal cord impulses pass from the terminal ramifications of central fibers to the intramedullary terminals of the dorsal root fibers. This involves the assumption of a synapse between axon and axon which is contrary to our present notions of the organization of the nervous system. It is significant that this is the one part of the theory which has not in its support a single well-established fact. The attempts made by Bayliss (10) and by Fofanow and Tschalussow (11) to demonstrate reflex antidromic vasodilatation by central stimulation of the vagus nerve certainly did not lead to conclusive results. New and more convincing evidence must be presented before this part of the theory can be accepted.

In the first place, it is not necessary to assume that the sensory fibers take any part in a true reflex vasodilatation. The important function which these fibers serve in the local vascular reaction of inflammation is sufficient to account for their having branches supplying the blood vessels. If the sensory fibers possess side branches capable of serving as vasodilators to the blood vessels and normally taking part in the inflammatory reaction caused by irritants, then it would follow that an artificial stimulus applied to the dorsal root would produce vasodilatation even though dilator impulses never left the spinal cord by this route.

But assuming that dilator impulses do leave the spinal cord and travel by way of the sensory fibers to the blood vessels, there exist possibilities of the synaptic interruption of the vasodilator path in the spinal ganglion which have never been taken into consideration. It is known that fine myelinated and unmyelinated fibers enter the spinal ganglion and after branching repeatedly form pericellular baskets about the ganglion cells. These baskets resemble very closely those formed by the terminal ramifications of the preganglionic fibers in the sympathetic ganglia and the fibers entering the spinal ganglion to form these pericellular networks resemble preganglionic fibers. The structures in question were thought by Dogiel to be the terminals of sensory fibers from the viscera. No satisfactory evidence for this interpretation was presented by Dogiel. It is out of harmony with the observations of Cajal (20) and contrary to the findings of Langley (21). The evidence against the existence of visceral afferent fibers of this type has been summarized in detail by Ranson and Billingsley (22). Furthermore it has not even been demonstrated that the fibers which form the pericellular networks about the spinal ganglion cells come from the sympathetic system. Huber (23) recently reviewed the literature on this subject and concluded that "the evidence presented by Cajal, Dogiel, Retzius, Huber and others cannot be regarded as entirely conclusive, since it has not been determined that the fine medullated fibers or the unmedullated fibers which appear to enter the spinal ganglia from without and end in pericellular plexuses, are, in fact, the neuraxes of sympathetic neurons."

The possibility must not be overlooked that these pericellular baskets which so closely resemble those of the sympathetic ganglia may be the terminal arborizations of preganglionic vasodilator fibers, in which case the sensory fibers with their side branches to the blood vessels would serve the purpose of relaying the impulses to these vessels. Under these circumstances an artificial stimulus applied to the sensory fibers in the dorsal roots would cause vasodilatation although normally dilator impulses would not leave the cord by these fibers. There are none of the established facts of antidromic conduction or of axon-reflex vasodilatation which are inconsistent with this theory.

There are three possible routes by which preganglionic vasodilator fibers might leave the spinal cord—none of which can as yet be entirely excluded. In the first place, these preganglionic fibers might leave the cord by way of the dorsal roots and end in the pericellular plexuses of the spinal ganglia. Bayliss did not exclude this possibility although he did show that the vasodilator fibers do not run from the spinal cord

through the spinal ganglia without interruption. Nicotine applied to the spinal ganglion or section of the dorsal roots and degeneration of the preganglionic fibers would not under these conditions affect the results of stimulation of the dorsal roots because the central branches of the dorsal root fibers would when stimulated be able to carry impulses through the ganglion and along the nerve to the blood vessels.

Or the preganglionic fibers under discussion might leave the spinal cord by way of the thoracic and upper lumbar ventral roots, pass through the white rami to the sympathetic trunk, descend in this trunk and then turn back through grey rami to the spinal ganglia. In this case they would be intermingled with constrictor fibers and very difficult to demonstrate, because of the preponderant effect of the constrictors when both types are stimulated at the same time. For this reason the experiment by which Bayliss attempted to demonstrate the absence of vasodilator fibers in the sympathetic trunk is not at all conclusive.

A third possibility which has never been excluded is that vasodilator fibers from the sacral nerves may ascend through the sympathetic trunk and run to the spinal ganglia by way of grey rami.

In the first paper of this series we demonstrated that the depressor reflex from central vagus stimulation is eliminated by doses of nicotine of the same order of magnitude as those required to paralyze the vasoconstrictor neurons in the sympathetic ganglia. Part of the reflex drop in blood pressure is believed to be due to active vasodilatation, and the remainder to the inhibition of vasoconstrictor tone. Whatever active vasodilatation occurs in the depressor reflex is certainly obliterated by nicotine; and this indicates that all of the vasodilator impulses which leave the central nervous system pass through a synapse of sympathetic character. Taking into consideration the facts of antidromic conduction and of axon-reflex vasodilatation, the most likely situations for such synaptic interruptions are the spinal ganglia and the plexuses along the arteries. As indicated in a previous paragraph, our experiments with nicotine have excluded the possibility of such synapses in the periarterial plexuses or in fact anywhere at the periphery. This leaves the spinal ganglion as the most logical place to look for synaptic interruptions in the vasodilator path.

SUMMARY

To summarize this somewhat complex argument, we may say that we believe the evidence clearly demonstrates that the sensory fibers are capable of conducting antidromically impulses which cause vasodilatation and that they play an important rôle in the vascular reaction which

accompanies inflammation. It has not been demonstrated, however, that vasodilator impulses normally leave the cord by way of the sensory fibers of the dorsal roots. Such vasodilator impulses as play a part in the depressor reflex from central vagus stimulation pass through synapses of sympathetic character and are interrupted by suitable doses of nicotine. If then the sensory fibers conduct dilator impulses antidromically in the depressor reflex, it becomes necessary to locate synapses of sympathetic character through which these impulses pass. It has been demonstrated by the use of nicotine that such synapses are not present in the periarterial plexuses or anywhere at the periphery and the most probable location for them is the spinal ganglion. It is known that the spinal ganglion does contain axonic ramifications in the form of pericellular plexuses which closely resemble those found in the sympathetic ganglia. The possibility cannot be excluded that preganglionic vasodilator fibers may leave the spinal cord by way of the dorsal roots to end in the spinal ganglia or that they may reach these ganglia by way of the sympathetic from the ventral roots of thoracic or sacral nerves.

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VASODILATOR MECHANISMS

III. The Vasodilator Action of Nicotine

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Contribution no. 97 from the Anatomical Laboratory of the Northwestern University Medical School

Received for publication July 10, 1922

The evidence presented by Bruce and other investigators to show that the congestion of inflammation is due to an axon reflex mediated through the branches of the sensory nerve fibers has been summarized on page 399 of the preceding paper of this series. Bardy (1) found that the conjunctivitis caused by a drop of mustard oil in the eye was eliminated by suitable doses of nicotine administered intravenously or locally. He concluded that those branches of the sensory fibers which convey the impulses to the blood vessels do not end directly in the walls of the vessels but rather in connection with local autonomic neurons through which the impulses are relayed to the vessels.

But it is obvious that the results obtained by Bardy with nicotine might be explained on the basis of a hypothetical adrenalin-like action of nicotine on the endings of the vasoconstrictor nerve fibers. So far as I am able to learn, there is nothing in the literature to exclude the possibility of such an action. The experiments of Hoskins and Ranson (2) demonstrated that about half of the pressor effect of nicotine is due to action of that poison on the peripheral nervous system. Following the usual conception of the action of nicotine they concluded that the locus of this peripheral action was the sympathetic ganglia; but their experiments did not exclude the possibility of a direct stimulation of the vasoconstrictor nerve endings. The assumption of such an adrenalin-like action seemed to the present writers the easiest way of explaining Bardy's results and an attempt was made to test this hypothesis by the injection of nicotine directly into the arterial blood stream.

Technique. Dogs were anesthetized with ether and tracheotomized. An ether bottle was attached to the tracheal cannula. The abdomen

was opened through the linea alba, the left abdominal sympathetic trunk extirpated and the right internal iliac artery prepared for the injections according to the technique described below. After the abdominal incision had been closed arrangements were made for taking a blood pressure tracing from the carotid artery and a plethysmograph tracing from the left leg. Most of these experiments were completed before those recorded in the preceding paper were begun, and in all these earlier experiments the plethysmograph was adjusted to the leg by the usual rubber cuff and vaseline method. The experiment was repeated later on two dogs with the improved plethysmographic technique described on page 395 of the preceding paper.

On account of the hemorrhage and thrombosis that follows piercing the arterial wall with the hypodermic needle, it was necessary to develop a special technique for these injections. After opening the abdomen in the middle line the right external iliac artery was doubly ligated and cut between the ligatures. The right internal iliac was followed into the pelvis, doubly ligated, and cut as far from its origin as possible. The middle sacral artery and the lowest lumbar vessels of the right side were ligated. An opening was made in the right flank and the free end of the internal iliac artery drawn through it. The lower margin of the incision in the flank was stitched to the psoas muscle. The median abdominal incision was then closed. When everything was in readiness for recording the carotid blood pressure and the volume of the left leg, a clamp was placed on the right internal iliac artery some distance above the ligature and a large hypodermic needle, the point of which had been converted into a bulbous extremity, was tied into the artery. The part of the artery distal to the clamp was then washed out with 4 per cent sodium citrate solution. A syringe containing the solution to be injected was then attached and the pressure in the syringe and distal part of the artery raised above that of the general blood pressure. The clamp was then removed and the fluid forced slowly into the artery. The clamp was reapplied immediately after the injection to prevent any blood getting into the cannula.

When injected into the right internal iliac the fluid was forced along the common iliac artery to the bifurcation of the aorta and was then carried with the blood stream to the left leg. After the effect of the injection had been recorded it was necessary to inject a little salt solution in order to wash out the blind artery; otherwise when another drug was injected its action was likely to be complicated by the residue of the first drug in the artery.

Tests were made of the action of adrenalin, nitroglycerine, acetylcholine and nicotine. In each case the active substance was dissolved in normal salt solution in such concentration that 5 or 6 cc. constituted the desired dose. This amount of normal salt solution injected slowly into the artery is usually without effect on the volume of the leg but in a few dogs it caused a slight vasodilatation. If however an equal amount of distilled water is injected, it causes a considerable increase in the volume of the leg.

This technique affords an easy means of testing the action of any drug on the peripheral blood vessels and has the advantage that repeated injections may be made and the experiment continued as long as desired. The method is particularly useful in distinguishing between the central and peripheral action of certain drugs and is so easy that it might well be used for class demonstrations. When, for example, adrenalin is injected in this way it causes first a marked vasoconstriction of the left leg and later after reaching the general circulation it causes a marked rise in blood pressure. Nitroglycerine causes first a swelling of the leg and later a fall in blood pressure. In a single experiment the locus of action of a number of such drugs can be demonstrated in succession; and when, as in the case of nicotine, the peripheral and central action of the substance are in opposition both can be demonstrated in their correct time relationship in a single tracing (fig. 1).

The peripheral action of nicotine. As has already been said, we began these experiments with the idea that nicotine might have an adrenalin-like action on the vasocontrictor nerve endings. We were very much surprised, therefore, to find that when nicotine acts directly on the blood vessels it produces a marked vasodilatation. This is illustrated in figure 1. Within a few seconds after the injection of 1.5 mgm. of nicotine in 6 cc. of normal salt solution the plethysmograph recorded a rapid rise in the volume of the leg and the pulse waves in the tracing became much more evident. This tracing was taken by the cuff and vaseline method and there was evidently enough contriction to delay the return of the venous blood and the drop to normal in the plethysmographic curve. After considerable time had elapsed the nicotine, returning through the veins, reached the general circulation and there was a rise in arterial pressure and an increase in the depth of respiration.

As already stated, the sympathetic trunk was extirpated on the left side to prevent the action of nicotine on the ganglia which it contains. In some of the first experiments this was not done and the nicotine reaching these ganglia through the lower lumbar arteries caused enough

vasoconstriction to more than overcome the peripheral vasodilator action of this toxin. Since some of the injected substance finds its way into the lumbar arteries and reaches the abdominal sympathetic trunk it probably also reaches the spinal ganglia and lower part of the spinal cord. It was therefore necessary to exclude the possibility that the vasodilator action of nicotine might be due to stimulation of one or the other of these structures. In two dogs the femoral and obturator nerves were cut within the pelvis, and the sciatic nerve at its exit from

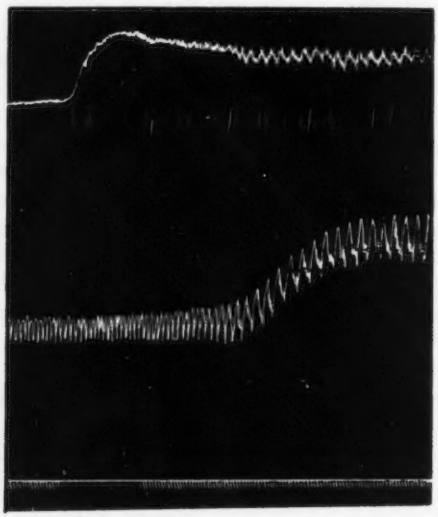

Fig. 1. Plethysmographic tracing of the leg above carotid blood pressure tracing from a dog, showing the effect of the injection of 1.5 mgm. of nicotine into the iliac artery. Old method.

the pelvis; and at the same time to eliminate any sympathetic fibers that might run along the blood vessels into the leg, the abdominal sympathetic trunk was extirpated. But these procedures did not in any way interfere with the vasodilator action of nicotine.

The tracing reproduced in figure 2 was taken from a dog in which all the nerves going to the leg had been cut. The improved method of setting the plethysmograph described on page 395 of the preceding paper was employed. Since there was no mechanical constriction of the

leg to delay the return of venous blood, the central action and resultant rise in blood pressure occurred much earlier than in the experiment represented in the preceding tracing, but for some reason not quite clear this rise in blood pressure was not so high as usual. A comparison of figures 1 and 2 gives a good idea of the difference in the quality of the results obtained by use of the two different methods of setting the plethysmograph.

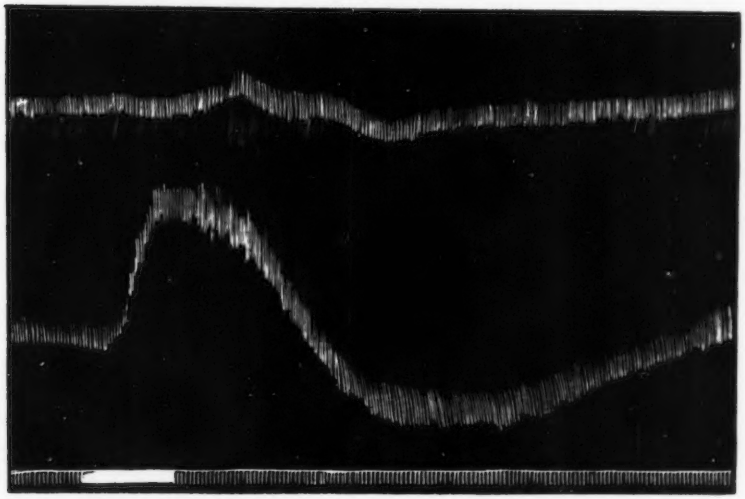

Fig. 2. Plethysmographic tracing of the leg below carotid blood pressure tracing from a dog, showing the effect of the injection of 1.5 mgm. of nicotine into the iliac artery. New method. Tracing retouched with white ink.

These experiments which were repeated on eighteen dogs show that nicotine has a peripheral action which is exactly opposite to that which it produces when it is able to reach the central nervous system and the sympathetic ganglia. When introduced into the general circulation it stimulates the vasoconstrictor center and the ganglia of the sympathetic trunk causing a generalized vasoconstriction and rise in arterial pressure. When introduced directly into the arteries of the leg it stimulates some local mechanism capable of causing vasodilatation. Doubtless when injected intravenously it stimulates the peripheral vasodilator as well as the central vasoconstrictor mechanism; but the latter being

the more powerful overcomes or at least masks the effect of the toxin on the other.

Even when all nerves to the leg have been cut and the abdominal sympathetic trunk removed the vasodilatation and swelling of the leg caused by the injection of nicotine are followed after a short interval by a fall in the volume of the limb below the original level. This is illustrated in figure 2. It cannot be due to the stimulation of the vasoconstrictor center or the sympathetic ganglia. It is equally evident in the majority of our other experiments but in them the possibility of the stimulation of sacral sympathetic ganglia was not excluded.

A second dose of nicotine has much less effect than the first and in some cases causes no local reaction in the blood vessels. It is unusual to get a vasodilatation from a third dose. This clearly shows that the local vasodilator mechanism which is stimulated by nicotine is also paralyzed by it. Since this toxin is known to stimulate and later paralyze sympathetic ganglion cells or the synapses associated with them one might explain its action on the peripheral blood vessels by assuming the presence in or upon the walls of these vessels of a vasodilator apparatus containing ganglion cells.

Our results seem to fit in perfectly with the hypothesis formulated by Bardy and summarized in a preceding paragraph. But, if the side branches of the sensory fibers going to the blood vessels are interrupted by sympathetic ganglion cells, as that author thought, it should be possible to eliminate the vasodilator action of the dorsal root fibers by suitable doses of nicotine. We were, therefore, much surprised to find that this is not the case. As stated in the preceding paper and illustrated by figures 1 and 2 on page 399, nicotine injected into the arterial blood stream going to the leg does not eliminate or even decrease the vasodilatation resulting from stimulation of the dorsal roots. It is therefore clear that peripheral neurons associated with the sensory vasodilator fibers, as postulated by Bardy, do not exist.

It is possible of course that there may be some other peripheral vasodilator mechanism, containing nerve cells, other than that represented by the branches of the sensory nerve fibers going to the blood vessels. Histological evidence of ganglion cells in the nerve plexuses accompanying the blood vessels is however far from convincing except in the case of arteries in the thoracic, abdominal and cranial cavities. Müller and Glasser (3) were able to demonstrate ganglion cells in the adventitia of the internal carotid, aorta, renal and other thoracic and abdominal arteries but none in the arteries of the body wall and extremities.

Michailow (4) found ganglion cells upon the blood vessels of some organs (e.g., the heart and urinary bladder). The presence of such cells in the arteries of the dog's leg is denied by Lapinsky (5). Bethe (6), Leontowitsch (7) and others have described what they considered as nerve cells in connection with the nerve-nets on the peripheral arteries but it is probable that these are nothing more than sheath cells (8).

Another possibility which must be taken into consideration in looking for an explanation of the peripheral effect of nicotine is that it may act on the endothelial cells lining the capillaries and arterioles much as it does on ganglion cells. It has recently been demonstrated that the caliber of the capillaries is in large part independent of the state of contraction in the arterioles. Krogh (9) has shown that in the tongue of the frog mechanical, thermal and most chemical stimuli produce a relaxation of the walls of the capillaries and arterioles and that they can then become filled with blood at a very low pressure. Hooker (10) has shown that the capillaries are innervated by fibers from the cervical sympathetic and that stimulation of this trunk causes them to contract. It is probable that the endothelial cells are the active elements in these reactions (11).

If it were assumed that nicotine stimulated and later paralyzed the endothelial cells we might account in this way for its peripheral vasodilator action. There is some evidence that it does have such an effect on the walls of the capillaries. Krogh showed that a drop of 0.5 per cent solution of nicotine on the tongue of the frog causes a marked capillary dilatation and a 0.1 per cent solution in a reagent basin has the same effect. Even by a dilution of this solution to about one-thousandth the dilator effect is not completely abolished.

There are however certain objections to this explanation for the vasodilator action of nicotine as illustrated by our tracings. The rise in volume of the leg is so abrupt that it would seem more easily explained as due to a relaxation of muscle fibers in the arterial wall. A marked increase in the pulse accompanies the swelling of the leg and this we are inclined to think indicates an arterial dilatation. We judge from the absence of any mention of a pulse wave in the papers of Hooker and Krogh that these waves have disappeared before the finer arterioles and capillaries have been reached (see also p. 397 of the preceding paper). If this is the case a large increase in the pulse wave in the plethysmograph tracing would indicate a dilatation of the arteries and larger arterioles.

It will be obvious, therefore, that we are not in position at the present time to give a satisfactory explanation of the vasodilator action of nicotine. It is clear however that the action is a specific one on a definite local mechanism. For after this mechanism has been paralyzed by repeated doses of that toxin the dilator action of nitroglycerine and acetylcholin and the constrictor action of adrenalin remain as good as ever. This shows that in the doses given nicotine does not injure the musculature of the arteries nor the nerve endings of the constrictor fibers. We may also say positively that nicotine does not act primarily on the same mechanism as nitroglycerine and acetylcholin.

The vasodilator action of acetylcholin. Reid Hunt (12) and Dale (13) have shown that acetylcholin is the most powerful circulatory depressant yet discovered. Perfusion experiments on the ear of the rabbit demonstrate that an exceedingly minute quantity of this substance is able to produce marked dilatation of the blood vessels and an increased flow of venous blood. This action must be a local one on the blood vessels themselves or on some nervous mechanism in their walls. According to Hunt, it acts on some as yet unknown vasodilator mechanism since it can be shown that it does not produce its effect through the "parasympathetic," "sympathetic" or dorsal root vasodilator fibers. Dale and Richards (14) suggest that acetylcholin may act directly on the smooth muscle of the arterial wall. The question naturally arose whether nicotine acted on the same mechanism and it seemed desirable to compare the vasodilator action of these two substances. Acetylcholin is difficult to obtain and Doctor Hunt very kindly furnished me with the material for these experiments.

When 5 cc. of physiological salt solution containing one part in 2,500,000 of acetycholin were injected into the right iliac artery there occurred a prompt and marked swelling of the left leg—and a greatly increased amplitude of the pulse wave in the plethysmograph tracing. The same reaction could be obtained many times in succession without diminishing effect. The time relations of the accompanying tracing (fig. 3) show that the vasodilatation is entirely peripheral in origin. It occurs promptly during the injection and is accompanied by a sharp fall in blood pressure due to the sudden decrease in the resistance in the arteries of the leg and pelvis. This is however quickly compensated by other factors in the circulation and the blood pressure returns to normal while the vasodilatation in the left leg is still at its height. By this time however the drug has completed the vascular circuit as is shown by a second drop in blood pressure due to a generalized vascular

dilatation. This drop is not very great because most of the drug has already been hydrolyzed in the blood. Nevertheless this second drop is not accompanied by a secondary rise in the plethysmograph as would be the case if a vasodilator center was being stimulated but by a rather rapid decrease in the volume of the leg.

In another experiment in which all the nerves going to the leg had been divided and the improved setting of the plethysmograph employed, essentially the same results were obtained although a much larger dose

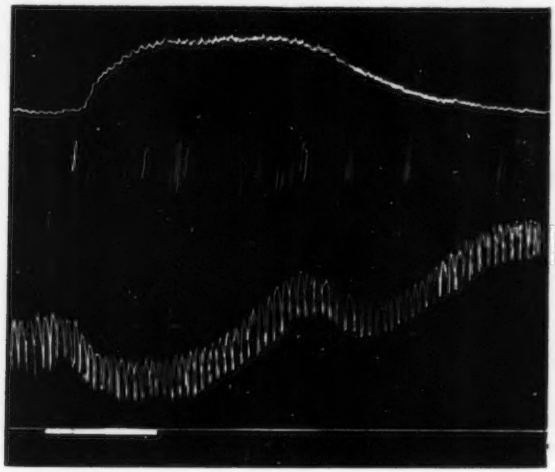

Fig. 3. Plethysmographic tracing of the leg above carotid blood pressure tracing from a dog, showing the effect of the injection of 1/500 mgm. of acetylcholin into the iliac artery. Old method.

was given. The intra-arterial injection of 5 cc. of normal salt solution containing one part in 200,000 of acetylcholin immediately caused a marked vasodilatation in the leg and secondarily a moderate fall in blood pressure (fig. 4). After about 20 seconds the drug reached the general circulation causing a generalized vasodilatation and a sharper fall in blood pressure which was followed passively by the plethysmographic curve. As the blood pressure returned toward normal the volume of the leg again increased far above the original level and the amplitude of the pulse wave indicated an enormous vasodilatation.

Our experiments confirm the conclusions reached by Hunt that the vasodilator action of acetylcholin is readily eliminated by atropin and is not affected by nicotine. After repeated arterial injections of nicotine have paralyzed the vasodilator mechanism upon which that toxin acts the effect of acetylcholin remains undiminished. On the other hand, the doses of atropin which eliminate the effect of acetylcholin have no influence on the dilator action of nicotine. We may safely conclude therefore that the two substances act on different mechanisms.

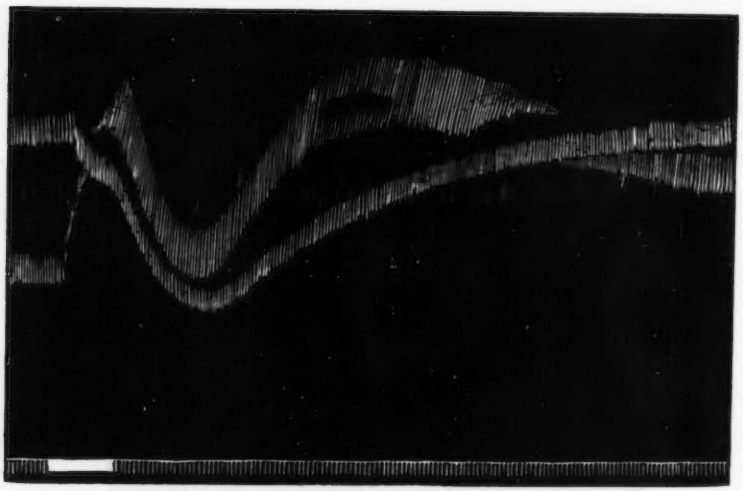

Fig. 4. Plethysmographic tracing of the leg below carotid blood pressure tracing from a dog, showing the effect of the injection of 1/40 mgm. of acetylcholin into the iliac artery. New method. Tracing retouched with white ink.

SUMMARY

When nicotine is injected into the arterial blood stream going to the leg it causes marked vasodilatation and swelling of the leg. Since this occurs when all nerve fibers running to the limb from the spinal cord and sympathetic trunk have been severed, it must be peripheral in origin. When the toxin is injected in the ordinary manner its action on the vasoconstrictor center and the sympathetic ganglia causes a general vasoconstriction which overcomes or at least effectively masks the peripheral vasodilator action.

It is not possible to say on what peripheral mechanism nicotine acts. Whatever this mechanism may be it is first stimulated and later paralyzed by that toxin. Two possibilities have been suggested. It may act on hypothetical ganglion cells belonging to a peripheral vasodilator mechanism, but if so these cells cannot be identified with those postulated by Bardy. Or it may act on the endothelium of the capillaries and arterioles. That the action is specific is shown by the fact that after repeated doses of nicotine have paralyzed this dilator mechanism nitroglycerine, acetylcholin and adrenalin still produce their characteristic vascular changes.

The work of Dale and Hunt showing that acetylcholin is a powerful vasodilator and that its effect is eliminated by atropin but not by nicotine is confirmed.

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